THERAPEUTIC APPLICATIONS OF LAMININ AND LAMININ-DERIVED PROTEIN FRAGMENTS

This is a continuation of US Application No. 08/947,057 filed 10/08/1997, which claims priority to US Provisional Application No. 60/027,981 filed 10/08/1996.

TECHNICAL FIELD

The invention relates to the discovery, identification and use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as well as related peptides and antibodies, for the therapeutic intervention and diagnosis of Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of an Alzheimer's beta-amyloid protein (Aß) specific binding region within the globular domain repeats of the laminin A chain, has led to new diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses which are disclosed.

BACKGROUND OF THE INVENTION

Alzheimer's disease is characterized by the accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein or Aß, in a fibrillar form, existing as extracellular amyloid plaques and as amyloid within the walls of cerebral blood vessels. Fibrillar Aß amyloid deposition in Alzheimer's disease is believed to be detrimental to the patient and eventually leads to toxicity and neuronal cell death, characteristic hallmarks of Alzheimer's disease. Accumulating evidence now implicates amyloid as a major causative factor of Alzheimer's disease pathogenesis. Discovery and identification of new compounds, agents, proteins, polypeptides or protein-derivatives as potential therapeutic agents to arrest Alzheimer's disease Aß amyloid formation, deposition, accumulation and/or persistence is desperately sought.

It is known that Aß is normally present in human blood and cerebrospinal fluid.

However, it is not known why this potential fibrillar protein remains soluble in circulating biological fluids. Can the agent(s) responsible for this extraordinary solubility of fibrillar Aß be applied to diagnostic and therapeutic regimens against the fibrillar Aß amyloid present in Alzheimer's brain?

SUMMARY OF THE INVENTION

The present invention provides answers to these questions and relates to the novel and surprising discovery that laminin and specific laminin-derived protein fragments are indeed potent inhibitors of Alzheimer's disease amyloidosis, and therefore have potential use for the therapeutic intervention and diagnosis of the amyloidoses. In addition, we have identified a specific region within laminin which interacts with the Alzheimer's disease beta-amyloid protein and contributes to the observed inhibitory and therapeutic effects. In addition, specific laminin-derived protein fragments which also interact with the Aß of Alzheimer's disease have been discovered to be present in human serum and cerebrospinal fluid, and implicate diagnostic applications which are described.

Laminin is a specific basement membrane component that is involved in several fundamental biological processes, and may play important roles in the pathogenesis of a number of different human diseases. Using a solid phase binding immunoassay, the present invention determined that laminin binds the Aß of Alzheimer's disease with a single binding constant of $K_d = 2.7 \times 10^{-9}$ M. In addition, using a Thioflavin T fluorometry assay (which quantitatively determines the amount of fibrillar amyloid formed), the present invention has determined that laminin is surprisingly an extremely potent inhibitor of Aß fibril formation. In this latter study, 25 µM of Aß (residues 1-40) was incubated at 37°C for 1 week in the

presence or absence of 100 nM laminin. Laminin was found to significantly ($p<0.001$) inhibit Aß (1-40) amyloid fibril formation by 2.9-fold at 1 hour, 4.6-fold at 1 day, 30.6-fold at 3 days and 27.1-fold at 1 week. Other basement membrane components including perlecan, fibronectin and type IV collagen were not effective inhibitors of Aß (1-40) fibrillogenesis in comparison to laminin, demonstrating the specificity of the inhibitory effect exhibited by laminin. The inhibitory effects of laminin on Aß fibrillogenesis was also found to occur in a dose-dependent manner. In addition, laminin was found to cause dissolution of pre-formed Alzheimer's disease amyloid fibrils in a dose-dependent manner following 4 days of incubation. Laminin was digested with V8, trypsin or elastase to determine small protease-resistent fragments of laminin which still interacted with Aß. A ~55 kilodalton (kDa) laminin fragment derived from V8 or elastase digested laminin was found to interact with biotinylated Aß (1-40). Amino acid sequencing of the ~55 kDa fragment identified an Aß-binding domain within laminin situated within the globular repeats of the laminin A chain.

Intact laminin was found to be present in human serum but not human cerebrospinal fluid, whereas laminin protein fragments ranging from ~120 kDa to ~200 kDa were found to be present in both human serum and cerebrospinal fluid. Of all the laminin protein fragments present in human biological fluids described above, a prominent ~130 kilodalton band was found in human serum and cerebrospinal fluid which primarily interacted with Aß as determined by ligand blotting methodology. This ~130 kilodalton laminin fragment is known as the E8 fragment (i.e. generated following elastase digestion of laminin)(Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993) and is also believed to consist of the globular domains of the laminin A chain. The interaction of specific laminin fragments such as the newly discovered ~130 kDa protein is believed to bind Aß in biological fluids and keep it in a soluble state. The present invention describes

the use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides for the therapeutic intervention and diagnosis of Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of a specific Alzheimer's Aß-binding region within the globular domain repeats of the laminin A chain, and the discovery of the presence of laminin fragments containing this region in human serum and cerebrospinal fluid, has led to new diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses.

FEATURES OF THE INVENTION

A primary object of the present invention is to establish new therapeutic methods and diagnostic applications for the amyloid diseases. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease and Down's syndrome (wherein the specific amyloid is referred to as beta-amyloid protein or Aß), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (wherein the specific amyloid is referred to as AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell dyscrasias (wherein the specific amyloid is referred to as AL amyloid), the amyloid associated with type II diabetes (wherein the specific amyloid is referred to as amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (wherein the specific amyloid is referred to as PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (wherein the specific amyloid is referred to as $beta_2$-microglobulin amyloid), the amyloid associated with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (wherein the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (wherein the specific amyloid is referred to as variants of procalcitonin).

A primary object of the present invention is to use laminin, laminin-derived protein fragments and/or laminin-derived polypeptides as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. "Laminin fragments, laminin-derived fragments, laminin-derived protein fragments and/or laminin-derived polypeptides", may include, but are not limited to, laminin A (or A1) chain, laminin B1 chain, laminin B2 chain, laminin A2 chain (merosin), laminin G1 chain, the globular domain repeats within the laminin A1 chain, SEQ ID NO: 1 (11 amino acid sequence within the mouse laminin A chain), SEQ ID NO: 2 (fourth globular repeat with the mouse laminin A chain), SEQ ID NO: 3 (fourth globular repeat within the human laminin A chain), SEQ ID NO: 4 (mouse laminin A chain), SEQ ID NO: 5 (human laminin A chain), SEQ ID NO: 6 (human laminin B1 chain), SEQ ID NO: 7 (mouse laminin B1 chain), SEQ ID NO: 8 (rat laminin B2 chain), SEQ ID NO: 9 (human laminin B2 chain), SEQ ID NO: 10 (mouse laminin G1 chain), SEQ ID NO: 11 (human laminin G1 chain), and all fragments or combinations thereof.

Yet another object of the present invention is to use conformational dependent proteins, polypeptides, or fragments thereof for the treatment of Alzheimer's disease and other amyloidoses. Such conformational dependent proteins include, but are not limited to, laminin, laminin-derived fragments including laminin A1 chain (SEQ ID NO 4; SEQ ID NO: 5), the globular repeat domains within the laminin A1 chain (SEQ ID NO: 2, SEQ ID NO:3), an 11- amino acid peptide sequence within the globular domain of the laminin A chain (SEQ ID NO:1), laminin B1 chain (SEQ ID NO:6, SEQ ID NO: 7), laminin B2 chain (SEQ ID NO: 8, SEQ ID NO:9), laminin G1 chain (SEQ ID NO: 10, SEQ ID NO: 11) and/or portions thereof.

Yet another aspect of the present invention is to use peptidomimetic compounds modelled from laminin, laminin-derived protein fragments and/or laminin-derived polypeptides, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Yet another object of the present invention is to mimic the 3-dimensional Aß-binding site(s) on laminin, laminin-derived protein fragments and/or laminin-derived polypeptides and use these mimics as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Yet a further aspect of the present invention is to use anti-idiotypic antibodies to laminin, laminin-derived protein fragments and/or laminin-derived polypeptides as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Another aspect of the invention is to provide new and novel polyclonal and/or monoclonal peptide antibodies which can be utilized in a number of in vitro assays to specifically detect Aß-binding laminin derived protein fragments and/or Aß-binding laminin derived polypeptides in human tissues and/or biological fluids. Polyclonal or monoclonal antibodies that are made specifically against a peptide portion or fragment of laminin which interacts with Aß can be utilized to detect and quantify amyloid disease specific laminin fragments in human tissues and/or biological fluids. These antibodies can be made by administering the peptides in antigenic form to a suitable host. Polyclonal or monoclonal

antibodies may be prepared by standard techniques known to those skilled in the art.

Another object of the present invention is to use laminin, the Aß-binding laminin fragments and/or laminin-derived polypeptides referred to above, for the detection and specific localization of laminin peptides important in the amyloid diseases in human tissues, cells, and/or cell culture using standard immunohistochemical techniques.

Yet another aspect of the present invention is to use antibodies recognizing laminin, any of the Aß-binding laminin fragments, and/or laminin-derived polypeptides including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, for in vivo labelling; for example, with a radionucleotide, for radioimaging to be utilized for in vivo diagnosis, and/or for in vitro diagnosis.

Yet another aspect of the present invention is to make use of laminin, Aß-binding laminin protein fragments and/or Aß-binding laminin-derived polypeptides including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potential therapeutics to inhibit the deposition, formation, and accumulation of fibrillar amyloid in Alzheimer's disease and other amyloidoses (described above), and to enhance the clearance and/or removal of preformed amyloid deposits in brain (for Alzheimer's disease and Down's syndrome amyloidosis) and in systemic organs (for systemic amyloidoses) .

Another object of the present invention is to use Aß-binding laminin-derived

polypeptides or fragments thereof, in conjunction with polyclonal and/or monoclonal antibodies generated against these peptide fragments, using in vitro assays to detect amyloid disease specific autoantibodies in human biological fluids. Specific assay systems can be utilized to not only detect the presence of autoantibodies against Aß-binding laminin-derived protein fragments or polypeptides thereof in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin protein fragments and/or laminin-derived polypeptide autoantibody levels.

Another aspect of the invention is to utilize laminin, laminin-derived protein fragments and/or laminin-derived polypeptide antibodies and/or molecular biology probes for the detection of these laminin derivatives in human tissues in the amyloid diseases.

Yet another object of the present invention is to use the laminin-derived protein fragments of the present invention in each of the various therapeutic and diagnostic applications described above. The laminin-derived protein fragments include, but are not limited to, the laminin A1 chain, the globular repeats within the laminin A1 chain, the laminin B1 chain, the laminin B2 chain, the laminin G1 chain, the laminin A2 chain (also known as merosin), and all constituents or variations thereof, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, including peptides which have at least 70% homology to the sequences disclosed herein. Specific laminin-derived protein fragments or peptides as described above may be derived from any species including, but are not limited to, human, murine, bovine, porcine, and/or equine species.

Another object of the invention is to provide polyclonal and/or monoclonal peptide

antibodies which can be utilized in a number of in vitro assays to specifically detect laminin protein fragments in human tissues and/or biological fluids. Polyclonal or monoclonal antibodies made specifically against a peptide portion or fragment of any of the laminin fragments described herein can be utilized to detect and quantify laminin-derived protein fragments in human tissues and/or biological fluids. A preferred embodiment is a polyclonal antibody made to the ~130 kilodalton Aß-binding laminin fragment present in human serum and cerebrospinal fluid. These antibodies can be made by isolating and administering the laminin-derived fragments and/or polypeptides in antigenic form to a suitable host. Polyclonal or monoclonal antibodies may be prepared by standard techniques by one skilled in the art.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in brain by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence, extent and/or progression of Alzheimer's disease and/or other brain amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in systemic organs by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in type II diabetes by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in other systemic amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to make use of peptides or fragments of laminin as described herein, including but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potential blocking therapeutics for the interaction of laminin and laminin-derived fragments in a number of biological processes and diseases (such as in Alzheimer's disease and other amyloid diseases described herein).

Yet another object of the invention is to utilize specific laminin-derived fragment antibodies, as described herein, for the detection of these laminin fragments in human tissues in the amyloid diseases.

Another object of the present invention is to use laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as described herein, for the treatment of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and

other amyloidoses.

Another object of the present invention is to use pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions,and sterile packaged powders, which contain laminin, laminin-derived protein fragments, and laminin-derived polypeptides, including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, to treat patients with Alzheimer's disease and other amyloidoses.

Yet another object of the present invention is to use laminin, laminin-derived protein fragments, and laminin-derived polypeptides, including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potent agents which inhibit amyloid formation, amyloid deposition, amyloid accumulation, amyloid persistence, and/or cause a dissolution of pre-formed or pre-deposited amyloid fibrils in Alzheimer's disease, and other amyloidoses.

Yet another object of the present invention is to provide the use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as described herein, for inhibition of amyloid formation, deposition, accumulation, and/or persistence, regardless of its clinical setting.

Yet another object of the present invention is to provide compositions and methods

involving administering to a subject a therapeutic dose of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, which inhibit amyloid deposition, including but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The proteins or polypeptides of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to amyloidosis. The methods of the invention are based, at least in part, in directly inhibiting amyloid fibril formation, and/or causing dissolution of preformed amyloid fibrils.

Yet another object of the present invention is to provide pharmaceutical compositions for treating amyloidosis. The pharmaceutical compositions include a therapeutic compound of the invention in an amount effective to inhibit amyloid deposition and a pharmaceutically acceptable vehicle.

These and other features and advantages of the present invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention.

FIGURE 1 is a binding curve demonstrating the binding interaction of EHS laminin

to substrate bound Aß (1-40). A single binding site with a $K_d = 2.7 \times 10^{-9}$ M is determined.

FIGURE 2 demonstrates the potent inhibition of Aß amyloid fibril formation by laminin as determined by a Thioflavin T fluorometry assay over a 1 week experimental period.

FIGURE 3 compares the potent inhibition of Aß amyloid fibril formation by laminin to other basement membrane components including fibronectin, type IV collagen and perlecan. Only laminin is found to have a potent inhibitory effect on Aß fibrillogenesis as early as 1 hour after incubation.

FIGURE 4 is a graph of a 1 week Thioflavin T fluorometry assay utilized to determine the potential dose-dependent effects of laminin on inhibition of Aß amyloid fibril formation. Significant dose-dependent inhibition of Aß (1-40) amyloid fibril formation is observed at 1 day, 3 days and 1 week of treatment with increasing concentrations of laminin.

FIGURE 5 is a graph of a Thioflavin T fluorometry assay utilized to determine the potential dose-dependent effects of laminin on dissolution of pre-formed Aß (1-40) amyloid fibrils within a 4 day incubation period. Laminin causes dissolution of pre-formed Aß amyloid fibrils in a dose-dependent manner.

FIGURE 6 is a graph of a 1 week Thioflavin T fluorometry assay utilized to determine the effects of laminin on islet amyloid polypeptide (amylin) fibrillogenesis, and determine whether laminin causes a dose-dependent inhibition of amylin fibril formation. Laminin does not significantly inhibit amylin fibrillogenesis suggesting its specificity for

Alzheimer's disease amyloidosis.

FIGURE 7 is a black and white photograph of laminin digested with V8 protease, separated by SDS-PAGE and following interaction with biotinylated Aß (1-40). The smallest fragment of V8-resistent laminin that interacts with Aß is a ~55 kilodalton fragment.

FIGURE 8 is a black and white photograph of laminin digested with trypsin, separated by SDS-PAGE and following interaction with biotinylated Aß (1-40). The smallest fragment of trypsin-resistent laminin that interacts with Aß is a ~30 kilodalton fragment.

FIGURE 9 is a black and white photograph of laminin digested with elastase, separated by SDS-PAGE and following interaction with biotinylated Aß (1-40). A ~55 kilodalton laminin fragment (arrow) that binds biotinylated Aß was identified and sequenced. Note also the presence of a ~130 kDa fragment (arrowheads) that binds Aß following 1.5 hours of elastase digestion (lane 2). Panel A is a ligand blot using biotinylated Aß as a probe, whereas panel B is Coomassie blue staining of the same blot in Panel A to locate the specific band(s) for sequencing.

a (Seq. ID 12)

FIGURE 10 shows the complete amino acid sequence of the mouse laminin A chain. Sequencing of the ~55 kilodalton Aß-binding band shown in Figure 9 leads to the identification of an 11 amino acid segment (underline and arrowhead) within the laminin A chain. This Aß binding region of laminin is situated within the globular domain repeats of the laminin A chain.

FIGURE 11 shows schematic diagrams of laminin and the newly discovered "Aß-

binding region" of laminin (shown in left panel; between the two arrowheads) which is situated within the last three globular domains of the laminin A chain.

FIGURE 12 is a black and white photograph of a Western blot demonstrating the presence of laminin (arrowheads) and/or laminin-derived protein fragments (bands between the two arrows) in human serum (lanes 1-7; left side) and human cerebrospinal fluid (lanes 1-7; right side) obtained from Alzheimer's disease, type II diabetes and normal aged patients. A ~110-130 kilodalton range of laminin positive protein fragments (between the two arrows) is present in both human serum and cerebrospinal fluid, whereas intact laminin (arrowheads) is only present in serum but not in cerebrospinal fluid.

FIGURE 13 is a black and white photograph demonstrating that intact laminin (arrow) and a prominent ~130 kilodalton band (arrowhead) present in human Alzheimer's disease, type II diabetes and normal aged patient serum, bind Aß. The Aß-binding laminin and specific Aß-binding laminin fragments in human serum were identified following separation by SDS-PAGE and interaction with nanomolar concentrations of biotinylated Aß (1-40).

FIGURE 14 is a black and white photograph demonstrating the presence of a prominent ~130 kilodalton band (arrow) in human Alzheimer's disease and normal aged patient cerebrospinal fluid, identified following separation by SDS-PAGE and following interaction with nanomolar concentrations of biotinylated Aß (1-40). This same ~130 kilodalton Aß-binding protein is also present in human serum (Figure 13).

DETAILED DESCRIPTION OF THE INVENTION

The following sections are provided by way of background to better appreciate the invention.

Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia in middle and late life, and is manifested by progressive impairment of memory, language, visuospatial perceptions and behavior (A Guide to the Understanding of Alzheimer's Disease and Related Disorders, edited by Jorm, New York University Press, New York 1987). A diagnosis of probable Alzheimer's disease can be made on clinical criteria (usually by the exclusion of other diseases, memory tests etc), but a definite diagnosis requires the histological examination of specific abnormalities in the brain tissue usually obtained at autopsy.

In Alzheimer's disease, the parts of the brain essential for cognitive processes such as memory, attention, language, and reasoning degenerate, robbing victims of much that makes us human, including independence. In some inherited forms of Alzheimer's disease, onset is in middle age, but more commonly, symptoms appear from the mid-60's onward. Alzheimer's disease is characterized by the deposition and accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein, Aß or ß/A4 (Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890, 1984; Masters et al, Proc. Natl. Acad. Sci. USA 82:4245-4249, 1985; Husby et al, Bull. WHO 71:105-108, 1993). Aß is derived from larger precursor proteins termed beta-amyloid precursor proteins (or ßPPs) of which there are several alternatively spliced variants. The most abundant forms of the ßPPs include

proteins consisting of 695, 751 and 770 amino acids (Tanzi et al, Nature 331:528-530, 1988; Kitaguchi et al, Nature 331:530-532, 1988; Ponte, et al, Nature 331:525-528, 1988). The small Aß peptide is a major component which makes up the amyloid deposits of neuritic "plaques" and in the walls of blood vessels (known as cerebrovascular amyloid deposits) in the brains of patients with Alzheimer's disease. In addition, Alzheimer's disease is characterized by the presence of numerous neurofibrillary "tangles", consisting of paired helical filaments which abnormally accumulate in the neuronal cytoplasm (Grundke-Iqbal et al, Proc. Natl. Acad. Sci. USA 83:4913-4917, 1986; Kosik et al, Proc. Natl. Acad. Sci. USA 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). The pathological hallmarks of Alzheimer's disease is therefore the presence of "plaques" and "tangles", with amyloid being deposited in the central core of plaques and within the blood vessel walls. It is important to note that a so-called "normal aged brain" has some amyloid plaques and neurofibrillary tangles present. However, in comparison, an Alzheimer's disease brain shows an over abundance of plaques and tangles. Therefore, differentiation of an Alzheimer's disease brain from a normal brain from a diagnostic point of view is primarily based on quantitative assessment of "plaques" and "tangles".

In an Alzheimer's disease brain, there are usually thousands of neuritic plaques. The neuritic plaques are made up of extracellular deposits consisting of an amyloid core usually surrounded by enlarged axons and synaptic terminals, known as neurites, and abnormal dendritic processes, as well as variable numbers of infiltrating microglia and surrounding astrocytes. The neurofibrillary tangles present in the Alzheimer's disease brain mainly consist of tau protein, which is a microtubule-associated protein (Grundke-Iqbal et al, Proc. Natl. Acad. Sci. USA 83:4913-4917, 1986; Kosik et al, Proc. Natl. Acad. Sci. USA 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). At the ultrastructural level, the tangle consists of paired helical filaments twisting like a ribbon, with a specific crossing

over periodicity of 80 nanometers. In many instances within a neurofibrillary tangle, there are both paired helical filaments and straight filaments. In addition, the nerve cells will many times die, leaving the filaments behind. These tangles are known as "ghost tangles" since they are the filamentous remnants of the dead neuron.

The other major type of lesion found in the brain of an Alzheimer's disease patient is the accumulation of amyloid in the walls of blood vessels, both within the brain parenchyma and in the walls of the larger meningeal vessels which lie outside the brain. The amyloid deposits localized to the walls of blood vessels are referred to as cerebrovascular amyloid or congophilic angiopathy (Mandybur, J. Neuropath. Exp. Neurol. 45:79-90, 1986; Pardridge et al, J. Neurochem. 49:1394-1401, 1987).

In addition, Alzheimer's disease patients demonstrate neuronal loss and synaptic loss. Furthermore, these patients also exhibit loss of neurotransmitters such as acetylcholine. Tacrine, the first FDA approved drug for Alzheimer's disease is a cholinesterase inhibitor (Cutler and Sramek, New Engl. J. Med. 328:808-810, 1993). However, this drug has showed limited success, if any, in the cognitive improvement in Alzheimer's disease patients and initially had major side effects such as liver toxicity.

For many years there has been an ongoing scientific debate as to the importance of "amyloid" in Alzheimer's disease and whether the "plaques" and "tangles" characteristic of this disease, were a cause or merely the consequences of the disease. Recent studies during the last few years have now implicated that amyloid is indeed a causative factor for Alzheimer's disease and not merely an innocent bystander. The Alzheimer's disease Aß protein in cell culture has been shown to cause degeneration of nerve cells within short periods of time (Pike et al, Br. Res. 563:311-314, 1991; J. Neurochem. 64:253-265,

1994). Studies suggest that it is the fibrillar structure, a characteristic of all amyloids, that is responsible for the neurotoxic effects. The Aß has also been found to be neurotoxic in slice cultures of hippocampus (the major memory region affected in Alzheimer's)(Harrigan et al, Neurobiol. Aging 16:779-789, 1995) and induces nerve cell death in transgenic mice (Games et al, Nature 373:523-527, 1995; Hsiao et al, Neuron 15:1203-1218, 1995). In addition, injection of the Alzheimer's Aß into rat brain causes memory impairment and neuronal dysfunction (Flood et al, Proc. Natl. Acad. Sci. U.S.A. 88:3363-3366, 1991; Br. Res. 663:271-276, 1994), two additional hallmarks of Alzheimer's disease. Probably, the most convincing evidence that amyloid (ie. beta-amyloid protein) is directly involved in the pathogenesis of Alzheimer's disease comes from genetic studies. It has been discovered that the production of Aß can result from mutations in the gene encoding, its precursor, known as the beta-amyloid precursor protein (Van Broeckhoven et al, Science 248:1120-1122, 1990; Europ. Neurol. 35:8-19, 1995; Murrell et al, Science 254:97-99, 1991; Haass et al, Nature Med. 1:1291-1296, 1995). This precursor protein when normally processed usually only produces very little of the toxic Aß. The identification of mutations in the amyloid precursor protein gene which causes familial, early onset Alzheimer's disease is the strongest argument that amyloid is central to the pathogenetic process underlying this disease. Four reported disease-causing mutations have now been discovered which demonstrate the importance of the beta-amyloid protein in causing familial Alzheimer's disease (reviewed in Hardy, Nature Genet. 1:233-234, 1992). All of these studies suggest that providing a drug to reduce, eliminate or prevent fibrillar Aß formation, deposition, accumulation and/or persistence in the brains of human patients should be considered an effective therapeutic.

Other Amyloid Diseases

The "amyloid diseases" consist of a group of clinically and generally unrelated human diseases which all demonstrate a marked accumulation in tissues of an insoluble extracellular substance known as "amyloid", and usually in an amount sufficient to impair normal organ function. Rokitansky in 1842 (Rokitansky, "Handbuch der pathologischen Anatomie", Vol. 3, Braumuller and Seidel, Vienna) was the first to observe waxy and amorphous looking tissue deposits in a number of tissues from different patients. However, it wasn't until 1854 when Virchow (Virchow, Arch. Path. Anat. 8:416, 1854) termed these deposits as "amyloid" meaning "starch-like" since they gave a positive staining with the sulfuric acid-iodine reaction, which was used in the 1850's for demonstrating cellulose. Although cellulose is not a constituent of amyloid, nonetheless, the staining that Virchow observed was probably due to the present of proteoglycans (PGs) which appear to be associated with all types of amyloid deposits. The name amyloid has remained despite the fact that Friederich and Kekule in 1859 discovered the protein nature of amyloid (Friedrich and Kekule, Arch. Path. Anat. Physiol. 16:50, 1859). For many years, based on the fact that all amyloids have the same staining and structural properties, lead to the postulate that a single pathogenetic mechanism was involved in amyloid deposition , and that amyloid deposits were thought to be composed of a single set of constituents. Current research has clearly shown that amyloid is not a uniform deposit and that amyloids may consist of different proteins which are totally unrelated (Glenner, N. England J. Med. 302:1283-1292, 1980).

Although the nature of the amyloid itself has been found to consist of completely different and unrelated proteins, all amyloids appear similar when viewed under the microscope due to amyloid's underlying protein able to adapt into a fibrillar structure. All

amyloids regardless of the nature of the underlying protein 1) stain characteristically with the Congo red dye and display a classic red/green birefringence when viewed under polarized light (Puchtler et al, J. Histochem. Cytochem. 10:355-364, 1962), 2) ultrastructurally consists of fibrils with a diameter of 7-10 nanometers and of indefinite length, 3) adopt a predominant beta-pleated sheet secondary structure. Thus, amyloid fibrils viewed under an electron microscope (30,000 times magnification) from the post-mortem brain of an Alzheimer's disease patient would look nearly identical to the appearance of amyloid present in a biopsied kidney from a rheumatoid arthritic patient. Both these amyloids would demonstrate a similar fibril diameter of 7-10 nanometers.

In the mid to late 1970's amyloid was clinically classified into 4 groups, primary amyloid, secondary amyloid, familial amyloid and isolated amyloid. Primary amyloid, is amyloid appearing de novo, without any preceding disorder. In 25-40% of these cases, primary amyloid was the antecedent of plasma cell dysfunction such as the development of multiple myeloma or other B-cell type malignancies. Here the amyloid appears before rather than after the overt malignancy. Secondary amyloid, appeared as a complication of a previously existing disorder. 10-15% of patients with multiple myeloma eventually develop amyloid (Hanada et al, J. Histochem. Cytochem. 19:1-15, 1971). Patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis can develop secondary amyloidosis as with patients with tuberculosis, lung abscesses and osteomyelitis (Benson and Cohen, Arth. Rheum. 22:36-42, 1979; Kamei et al, Acta Path. Jpn. 32:123-133, 1982; McAdam et al, Lancet 2:572-575, 1975). Intravenous drug users who self-administer and who then develop chronic skin abscesses can also develop secondary amyloid (Novick, Mt. Sin. J. Med. 46:163-167, 1979). Secondary amyloid is also seen in patients with specific malignancies such as Hodgkin's disease and renal cell carcinoma (Husby et al, Cancer Res. 42:1600-1603, 1982). Although these were all initially classified as secondary amyloid,

once the amyloid proteins were isolated and sequenced many of these turned out to contain different amyloid proteins.

The familial forms of amyloid also showed no uniformity in terms of the peptide responsible for the amyloid fibril deposited. Several geographic populations have now been identified with genetically inherited forms of amyloid. One group is found in Israel and this disorder is called Familial Mediterranean Fever and is characterized by amyloid deposition, along with recurrent inflammation and high fever (Mataxas, Kidney 20:676-685, 1981). Another form of inherited amyloid is Familial Amyloidotic Polyneuropathy, and has been found in Swedish (Skinner and Cohen, Biochem. Biophys. Res. Comm. 99:1326-1332, 1981), Portuguese (Saraiva et al, J. Lab. Clin. Med. 102:590-603, 1983; J. Clin. Invest. 74:104-119, 1984) and Japanese (Tawara et al, J. Lab. Clin. Med. 98:811-822, 1981) nationalities. Amyloid deposition in this disease occurs predominantly in the peripheral and autonomic nerves. Hereditary amyloid angiopathy of Icelandic origin is an autosomal dominant form of amyloid deposition primarily affecting the vessels in the brain, and has been identified in a group of families found in Western Iceland (Jennson et al, Clin. Genet. 36:368-377, 1989). These patients clinically have massive cerebral hemorrhages in early life which usually causes death before the age of 40.

The primary, secondary and familial forms of amyloid described above tend to involve many organs of the body including heart, kidney, liver, spleen, gastrointestinal tract, skin, pancreas, and adrenal glands. These amyloid diseases are also referred to as "systemic amyloids" since so many organs within the body demonstrate amyloid accumulation. For most of these amyloidoses, there is no apparent cure or effective treatment and the consequences of amyloid deposition can be detrimental to the patient. For example, amyloid deposition in kidney may lead to renal failure, whereas amyloid

deposition in heart may lead to heart failure. For these patients, amyloid accumulation in systemic organs leads to eventual death generally within 3 to 5 years.

Isolated forms of amyloid, on the other hand, tend to involve a single organ system. Isolated amyloid deposits have been found in the lung, and heart (Wright et al, Lab. Invest. 30:767-773, 1974; Pitkanen et al, Am. J. Path. 117:391-399, 1984). Up to 90% of type II diabetic patients (non-insulin dependent form of diabetes) have isolated amyloid deposits in the pancreas restricted to the beta cells in the islets of Langerhans (Johnson et al, New Engl. J. Med. 321:513-518, 1989; Lab. Invest. 66:522-535, 1992). Isolated forms of amyloid have also been found in endocrine tumors which secrete polypeptide hormones such as in medullary carcinoma of the thyroid (Butler and Khan, Arch. Path. Lab. Med. 110:647-649, 1986; Berger et al, Virch. Arch. A Path. Anat. Hist. 412:543-551, 1988). A serious complication of long term hemodialysis is amyloid deposited in the medial nerve and clinically associated with carpal tunnel syndrome (Gejyo et al, Biochem. Biophys. Res. Comm. 129:701-706, 1985; Kidney Int. 30:385-390, 1986). By far, the most common type and clinically relevant type of organ-specific amyloid, and amyloid in general, is that found in the brains of patients with Alzheimer's disease (see U.S. Patent No. 4,666,829 and Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890, 1984; Masters et al, Proc. Natl. Acad. Sci., USA 82:4245-4249, 1985). In this disorder, amyloid is predominantly restricted to the central nervous system. Similar deposition of amyloid in the brain occurs in Down's syndrome patients once they reach the age of 35 years (Rumble et al, New England J. Med. 320:1446-1452, 1989; Mann et al, Neurobiol. Aging 10:397-399, 1989). Other types of central nervous system amyloid deposition include rare but highly infectious disorders known as the prion diseases which include Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, and kuru (Gajdusek et al, Science 197:943-960, 1977; Prusiner et al, Cell 38:127-134, 1984; Prusiner, Scientific American 251:50-59, 1984;

Prusiner et al, Micr. Sc. 2:33-39, 1985; Tateishi et al, Ann. Neurol. 24:35-40, 1988).

It was misleading to group the various amyloidotic disorders strictly on the basis of their clinical features, since when the major proteins involved were isolated and sequenced, they turned out to be different. For example, amyloid seen in rheumatoid arthritis and osteoarthritis, now known as AA amyloid, was the same amyloid protein identified in patients with the familial form of amyloid known as Familial Mediterranean Fever. Not to confuse the issue, it was decided that the best classification of amyloid should be according to the major protein found, once it was isolated, sequenced and identified.

Thus, amyloid today is classified according to the specific amyloid protein deposited. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease, Down's syndrome and hereditary cerebral hemorrhage with amyloidosis of the Dutch type (wherein the specific amyloid is now known as the beta-amyloid protein or Aß), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell abnormalities (AL amyloid), the amyloid associated with type II diabetes (amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome ($beta_2$-microglobulin amyloid), the amyloid associated with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (prealbumin or transthyretin amyloid), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (variants of procalcitonin).

Laminin and Its Structural Domains

Laminin is a large and complex 850 kDa glycoprotein which normally resides on the basement membrane and is produced by a variety of cells including embryonic, epithelial and tumor cells (Foidart et al, Lab. Invest. 42:336-342, 1980; Timpl et al, Methods Enzymol. 82:831-838, 1982). Laminin-1 (is derived from the Engelbreth-Holm-Swarm tumor) and is composed of three distinct polypeptide chains, A, B1 and B2 (also referred to as alpha1, ß1 and gamma-1, respectively), joined in a multidomain structure possessing three shorts arms and one long arm (Burgeson et al, Matrix Biol. 14:209-211, 1994). Each of these arms is subdivided into globular and rodlike domains. Studies involving in vitro self-assembly and the analysis of cell-formed basement membranes have shown that laminin exists as a polymer, forming part of a basement membrane network (Yurchenco et al, J. Biol. Chem. 260:7636-7644, 1985; Yurchenco et al, J. Cell Biol. 117:1119-1133, 1992; Yurchenco and Cheng, J. Biol. Chem. 268: 17286-17299, 1993). Laminin is believed to play important roles in a number of fundamental biological processes including promotion of neural crest migration (Newgreen and Thiery, Cell Tissue Res. 211:269-291, 1980; Rovasio et al, J. Cell Biol. 96:462-473, 1983), promotion of neurite outgrowth (Lander et al, Proc. Natl. Acad. Sci. 82:2183-2187, 1985; Bronner-Fraser and Lallier, Cell Biol. 106:1321-1329, 1988), the formation of basement membranes (Kleinman et al, Biochem. 22:4969-4974, 1983), the adhesion of cells (Engvall et al, J. Cell Biol. 103: 2457-2465, 1986) and is inducible in adult brain astrocytes by injury (Liesi et al, EMBO J. 3:683-686, 1984). Laminin interacts with other components including type IV collagen (Terranova et al, Cell 22:719-726, 1980; Rao et al, Biochem. Biophys. Res. Comm. 128:45-52, 1985; Charonis et al, J. Cell Biol. 100: 1848-1853, 1985; Laurie et al, J. Mol. Biol. 189:205-216, 1986), heparan sulfate proteoglycans (Riopelle and Dow, Brain Res. 525:92-100, 1990; Battaglia et al, Eur. J. Biochem. 208:359-366, 1992) and heparin (Sakashita et al,

FEBS Lett. 116:243-246, 1980; Del Rosso et al, Biochem. J. 199:699-704, 1981; Skubitz et al, J. Biol. Chem. 263:4861-4868, 1988).

Several of the functions of laminin have been found to be associated with the short arms. First, the short arms have been found to participate in laminin polymerization (Yurchenco et al, J. Cell Biol. 117:1119-1133, 1992; Yurchenco and Cheng, J. Biol. Chem. 268: 17286-17299, 1993). A recently proposed three-arm interaction hypothesis of laminin polymerization (Yurchenco and Cheng, J. Biol. Chem. 268: 17286-17299, 1993) further holds that self-assembly is mediated through the end regions of each of the three short arms. A prediction of this model is that each short arm can independently and competitively inhibit laminin polymerization. However, it has not been possible to formally test this prediction using conventional biochemical techniques because of an inability to separate the alpha and gamma chains. Second, several heparin binding sites have been thought to reside in the short arms (Yurchenco et al, J. Biol. Chem. 265:3981-3991, 1990; Skubitz et al, J. Cell Biol. 115:1137-1148, 1991), although the location of these sites have remained obscure. Third, the alpha1ß1 integrin has been found to selectively interact with large short arm fragments containing all or most of the short arm domains (Hall et al, J. Cell Biol. 110:2175-2184, 1990; Goodman et al, J. Cell Biol. 113:931-941, 1991).

Most functional activities of laminin appear to be dependent upon the conformational state of the glycoprotein. Specifically, self-assembly and its calcium dependence, nidogen (entactin) binding to laminin, alpha6ß1 integrin recognition of the long arm, heparin binding to the proximal G domain (cryptic) and RGD-dependent recognition of the short A chain of laminin (cryptic) have all been found to be conformationally dependent (Yurchenco et al, J. Biol. Chem. 260:7636-7644, 1985; Fox et al, EMBO J. 10:3137-3146, 1991; Sung et al, J. Cell Biol. 123:1255-1268, 1993). Two consequences of improperly folded laminin, loss of

normal functional activity and the activation of previously cryptic activities, suggest that it is important to map and characterize biological activities using correctly folded laminin or conformational homologues to any particular laminin or laminin fragment.

Laminin may also be involved in the pathogenesis of a number of important diseases. For example, in diabetes significant decrease in the levels of laminin on the glomerular basement membranes indicates that a molecular imbalance occurs (Shimomura and Spiro, Diabetes 36:374-381, 1987). In experimental AA amyloidosis (ie. inflammation-associated amyloidosis), increased levels of laminin are observed at the sites of AA amyloid deposition (Lyon et al, Lab. Invest. 64:785-790, 1991). However, the role(s) of laminin in systemic amyloidosis is not known. In Alzheimer's disease and Down's syndrome, laminin is believed to be present in the vicinity of Aß-containing amyloid plaques (Perlmutter and Chui, Brain Res. Bull. 24:677-686, 1990; Murtomaki et al, J. Neurosc. Res. 32:261-273, 1992; Perlmutter et al, Micro. Res. Tech. 28:204-215, 1994).

Previous studies have indicated that the various isoforms of the beta-amyloid precursor proteins of Alzheimer's disease, bind both the basement membrane proteins perlecan (Narindrasorasak et al, J. Biol. Chem. 266:12878-12883, 1991) and laminin (Narindrasorasak et al, Lab. Invest. 67:643-652, 1992). With regards to laminin, it was not previously known whether laminin interacts with Aß, whether a particular domain of laminin (if any) participates in Aß interactions, and whether laminin had any significant role(s) in Aß amyloid fibrillogenesis.

The present invention has discovered that laminin binds Aß with relatively high affinity and surprisingly laminin is a potent inhibitor of Aß amyloid formation, and causes dissolution of pre-formed Alzheimer's disease amyloid fibrils. In addition, a 55-kilodalton

elastase resistent fragment of laminin which also binds Aß has been localized to the globular domain repeats within the A chain of laminin. This region is believed to be responsible for many of the inhibitory effects that laminin has on Alzheimer's disease amyloidosis. These findings indicate that laminin, laminin-derived protein fragments and/or laminin-derived polypeptides, particularly those containing the disclosed Aß-binding site within the globular domain repeats within the laminin A chain, may serve as novel inhibitors of Aß amyloidosis in Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of an Alzheimer's Aß-binding region within the globular domain repeats of the laminin A chain, and the discovery of its presence in human serum and cerebrospinal fluid, as a ~130 kDa laminin-derived fragment, leads to novel diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses.

Examples

The following examples are provided to disclose in detail preferred embodiments of the binding interaction of laminin with Aß, and the potent inhibitory effects of laminin and disclosed fragments on Aß fibril formation. However, it should not be construed that the invention is limited to these specific examples.

Example 1

Binding of Laminin to the Beta-Amyloid Protein (Aß) of Alzheimer's Disease

2 µg of Aß (1-40)(Bachem Inc., Torrance, CA USA; Lot #WM365) in 40 µl of Tris-buffered saline (TBS)(pH 7.0) was allowed to bind overnight at 4°C to microtiter wells (Nunc plates, Maxisorb). The next day all of the microtiter wells were blocked by

incubating with 300 µl of Tris-buffered saline containing 100 mM Tris-HCl, 50 mM NaCl, 0.05% Tween-20, and 3 mM NaN_3 (pH 7.4)(TTBS) plus 2% bovine serum albumin (BSA). Various dilutions (ie. 1:10, 1:30, 1:90, 1:270, 1:810, 1:2430 and 1:7290) of Engelbreth-Holm-Swarm (EHS) mouse tumor laminin (1 mg/ml)(Sigma Chemical Co., St. Louis, MO, USA) in 250 µl of TBS (pH 7.4) were placed in wells (in triplicate) either containing substrate bound Aß (1-40) or blank, and allowed to bind overnight at 4°C overnight. The next day, the wells were rinsed 3 times with TTBS, and then probed for 2 hours with 100 µl of rabbit anti-laminin antibody (Sigma Chemical Company, St. Louis, MO) diluted 1:10,000 in TTBS. After 3 rinses with TTBS, the wells were then incubated for 2 hours on a rotary shaker with 100 µl of secondary probe consisting of biotinylated goat anti-rabbit (1:1000) and strepavidin-peroxidase (1:500 dilution of a 2 µg/ml solution) in TTBS containing 0.1% BSA. The wells were then rinsed 3 times with TTBS and 100 µl of a substrate solution (OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO) was added to each well and allowed to develop for 10 minutes or until significant color differences were observed. The reaction was stopped with 50 µl of 4N H_2SO_4 and read on a Model 450 microplate reader (Biorad, Hercules, CA USA) at 490 nm. Data points representing a mean of triplicate determinations were plotted and the affinity constants (ie. K_d) were determined using Ultrafit (version 2.1, Biosoft, Cambridge, U.K.) as described below.

The binding data were analyzed assuming a thermodynamic equilibrium for the formation of the complex BL, from the laminin ligand in solution, L, and the uncomplexed Aß adsorbed to the microtiter well, B, according to the equation: $K_d = [B] \times [L]/[BL]$. We elected to determine K_d's by using an enzyme-linked immunoassay that gives a color signal that is proportional to the amount of unmodified laminin bound to Aß (Engel, J. and

Schalch, W., Mol. Immunol. 17:675-680, 1980; Mann, K. et al, Eur. J. Biochem. 178:71-80, 1988; Fox, J.W. et al, EMBO J. 10:3137-3146, 1991; Battaglia, C. et al, Eur. J. Biochem. 208:359-366, 1992).

To account for potential non-specific binding, control wells without Aß (in triplicate) were included for each concentration of laminin used in each binding experiment. Optical densities of the control wells never exceeded 0.050 at all laminin concentrations employed for these experiments. The optical densities of the control wells were subtracted from the optical densities of the Aß-containing wells that received similar laminin concentrations. Non-specific absorbance obtained from Aß containing wells that did not receive laminin were also subtracted from all data points. Thus, the equation in the form of: $OD_{exp}=OD_o + (S \times [laminin]) + (OD_{max} \times [laminin]/([laminin] + K_d)$ where (S x [laminin]) represents non-specific binding (control wells) and OD_o is the non-specific absorbance, becomes $OD_{exp} = OD_{max} \times [laminin]/([laminin] + K_d)$. Therefore, at 50 % saturation $OD_{exp} = 0.50\ OD_{max}$ and K_d = [laminin]. Determination of [laminin] at 50% saturation was performed by non-linear least square program (Ultrafit from Biosoft, UK) using a one-site model.

As demonstrated in Figure 1, EHS laminin bound immobilized Aß (1-40) with a single binding constant with an apparent dissociation constant of $K_d = 2.7 \times 10^{-9}$ M. Several repeated experiments utilizing this solid phase binding immunoassay indicated that laminin bound Aß (1-40) repetitively with one apparent binding constant.

Example 2

Inhibition of Alzheimer's Disease Aß Fibril Formation by Laminin

The effects of laminin on Aß fibrillogenesis was also determined using the previously described method of Thioflavin T fluorometry (Naiki et al, Lab. Invest. 65:104-110, 1991; Levine III, Protein Sci. 2:404-410, 1993; Levine III, Int. J. Exp. Clin. Invest. 2:1-6, 1995; Naiki and Nakakuki, Lab. Invest. 74:374-383, 1996). In this assay, Thioflavin T binds specifically to fibrillar amyloid and this binding produces a fluorescence enhancement at 480 nm that is directly proportional to the amount of amyloid fibrils formed (Naiki et al, Lab. Invest. 65:104-110, 1991; Levine III, Protein Sci. 2:404-410, 1993; Levine III, Int. J. Exp. Clin. Invest. 2:1-6, 1995; Naiki and Nakakuki, Lab. Invest. 74:374-383, 1996). In a first study, the effects of EHS laminin on Aß (1-40) fibrillogenesis was assessed. For this study, 25 µM of freshly solubilized Aß (1-40)(Bachem Inc., Torrance, CA, USA; Lot # WM365) was incubated in microcentrifuge tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of 100 nM EHS laminin (Sigma Chemical Company, St. Louis, MO, USA) in 100 mM Tris, 50 mM NaCl, pH 7.0 (TBS). 100 nM of laminin utilized for these studies represented a Aß:laminin molar ratio of 250:1. 50 µl aliquots were then taken from each tube for analysis at 1 hr, 1 day, 3 days, and 1 week. In a second set of studies, the effects of laminin on Aß (1-40) fibril formation was directly compared to other basement membrane components including fibronectin, type IV collagen and perlecan. For these studies, 25 µM of freshly solubilized Aß (1-40) was incubated in microcentrifuge tubes for 1 week (in triplicate) either alone, or in the presence of 100 nM of EHS perlecan (isolated as previously described)(Castillo et al, J. Biochem. 120:433-444, 1996), fibronectin (Sigma Chemical Company, St. Louis, MO, USA) or type

IV collagen (Sigma Chemical Company, St. Louis, MO, USA). 50 ul aliquots were then taken for analysis at 1 hour, 1 day, 3 days and 1 week. In a third set of studies, 25μM of freshly solubilized Aß (1-40) was incubated in microcentrifuge tubes for 1 week (in triplicate) either alone, or in the presence of increasing concentrations of laminin (i.e. 5 nM, 15 nM, 40 nM and 100 nM). 50 μl aliquots were taken for analysis at 1 hour, 1 day, 3 days and 1 week.

For each determination described above, following each incubation period, Aß peptides +/- laminin, perlecan, fibronectin or type IV collagen, were added to 1.2 ml of 100 μM Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50 mM phosphate buffer (pH 6.0). Fluorescence emission at 480 nm was measured on a Turner instrument-model 450 fluorometer at an excitation wavelength of 450 nm. For each determination, the fluorometer was calibrated by zeroing in the presence of the Thioflavin T reagent alone, and by seting the 50 ng/ml riboflavin (Sigma Chemical Co., St. Louis, Mo) in the Thioflavin T reagent to 1800 fluorescence units. All fluorescence determinations were based on these references and any background fluorescence given off by laminin, perlecan, type IV collagen, or fibronectin alone in the presence of the Thioflavin T reagent was always subtracted from all pertinent readings.

As shown in Figure 2, freshly suspended Aß (1-40) alone, following a 1 hour incubation at 37°C, demonstrated an initial fluorescence of 41 fluorescence units. During the 1 week incubation period there was a gradual increase in the fluorescence of 25 μM Aß (1-40) alone, increasing 6.7-fold from 1 hour to 1 week, with a peak fluorescence of 379 fluorescence units observed at 1 week. This increase was significantly inhibited when Aß (1-40) was co-incubated with laminin, in comparison to Aß alone. Aß (1-40) co-incubated with laminin displayed fluorescence values that were 2.9-fold lower ($p<0.001$) at 1 hour,

4.6-fold lower ($p<0.0001$) at 1 day, 30.6-fold lower ($p<0.0001$) at 3 days and 27.1-fold lower ($p<0.0001$) at 1 week. This study indicated that laminin was a potent inhibitor of Aß amyloid fibril formation, nearly completely inhibiting amyloid fibril formation even after 1 week of incubation.

To determine whether the inhibitory effects of laminin was specific to this basement membrane component, an direct comparison was made to other known basement membrane components including perlecan, fibronectin, and type IV collagen. In these studies 25 µM of Aß (1-40) was incubated in the absence or presence of either 100 nM of laminin, 100 nM of fibronectin, 100 nM of type IV collagen and 100 nM of perlecan (Figure 3). Freshly solubilized Aß (1-40) when incubated at 37°C gradually increased in fluorescence levels from 1 hour to 1 week (by 10.8-fold)(Figure 3), as previously demonstrated (Figure 2). Perlecan was found to significantly accelerate Aß (1-40) amyloid formation at 1 day and 3 days, whereas fibronectin and type IV collagen only showed significant inhibition of Aß (1-40) fibrillogenesis at 1 week. Laminin, on the other hand, was again found to be a very potent inhibitor of Aß fibrillogenesis causing a 9-fold decrease at 1 and 3 days, and a 21-fold decrease at 1 week. This study reconfirmed the potent inhibitory effects of laminin on Aß fibrillogenesis, and demonstrated the specificity of this inhibition, since none of the other basement membrane components (including fibronectin, type IV collagen and perlecan) were very effective inhibitors.

To determine whether the inhibitory effects of laminin on Aß fibrillogenesis occurred in a dose-dependent manner, different concentrations of laminin (i.e. 5nM, 15 nM, 40 nM and 100 nM) were tested. As shown in Figure 4, freshly solubilized Aß (1-40) when incubated at 37°C gradually increased from 1 hour to 1 week, as previously demonstrated (Figures 2 and 3). 100 nM of laminin significantly inhibited Aß fibril formation at all time

points studied, including 1 hour, 1 day, 3 days and 7 days. Laminin was also found to inhibit Aß fibril formation in a dose-dependent manner which was significant ($p<0.05$) by 3 days of incubation. At 3 days and 7 days, both 100 nM and 40 nM of laminin significantly inhibited Aß fibril formation. This study reconfirmed that laminin was a potent inhibitor of Aß fibril formation and that this inhibition occurred in a dose-dependent manner.

Example 3

Laminin Causes Dose-Dependent Dissolution of Pre-Formed Alzheimer's Disease Amyloid Fibrils

The next study was implemented to determine whether laminin was capable of causing a dose-dependent dissolution of pre-formed Alzheimer's disease Aß (1-40) amyloid fibrils. This type of activity would be important for any potential anti-Alzheimer's amyloid drug which can be used in patients who already have substantial amyloid deposition in brain. For example, Alzheimer's disease patients in mid-to late stage disease have abundant amyloid deposits in their brains as part of both neuritic plaques and cerebrovascular amyloid deposits. A therapeutic agent capable of causing dissolution of pre-existing amyloid would be advantageous for use in these patients who are at latter stages of the disease process.

For this study, 1 mg of Aß (1-40)(Bachem Inc., Torrance, CA, USA; Lot #WM365) was dissolved in 1.0 ml of double distilled water (1mg/ml solution) and then incubated at 37°C for 1 week. 25µM of fibrillized Aß was then incubated at 37°C in the presence or absence of laminin (from EHS tumor; Sigma Chemical Company, St. Louis, MO, USA) at concentrations of 125 nM, 63 nM, 31 nM and 16 nM containing 150 mM Tris HCl, 10 mM NaCl, pH 7.0. Following a 4 day incubation, 50 µl aliquots were added

to 1.2ml of 100μM Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50mM $NaPO_4$ (pH 6.0) for fluorometry readings as described in example 2.

As shown in Figure 5, dissolution of pre-formed Alzheimer's disease Aß amyloid fibrils by laminin occurred in a dose-dependent manner. A significant ($p<0.001$) 41% dissolution of pre-formed Aß amyloid fibrils was observed with 125 nM of laminin, whereas 63 nM of laminin caused a significant ($p<0.001$) 39% dissolution. Furthermore, 31 nM and 16 nM of laminin still caused a significant ($p<0.01$) 28% and 25% dissolution of pre-formed Aß amyloid fibrils.These data demonstrated that laminin causes dissolution of pre-formed Alzheimer's disease amyloid fibrils in a dose-dependent manner following a 4-day incubation.

Example 4

Laminin Does Not Significantly Inhibit Islet Amyloid Polypeptide (Amylin) Fibril Formation

In the next study, the specificity of the laminin inhibitory effects on Alzheimer's disease amyloid was determined by testing laminin's potential effects on another type of amyloid. Amyloid accumulation occurs in the islets of Langerhans in ~90% of patients with type II diabetes (Westermark et al, Am. J. Path. 127:414-417, 1987). The major protein in islet amyloid is a 37 amino acid peptide, termed islet amyloid polypeptide or amylin which is known to be a normal secretory product of the beta-cells of the pancreas (Cooper et al, Proc. Natl. Acad. Sci., 84:8628-8632, 1987). The dose-dependent effects of laminin on amylin fibrillogenesis was determined using the Thioflavin T fluorometry assay. 25 μM of Aß (1-40)(Bachem Inc., Torrance, CA, USA; Lot #WM365) was incubated in microcentrifuge

tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of 5 nM, 15 nM, 40 nM and 100 nM of laminin in 150 mM Tris HCl, 10 mM NaCl, pH 7.0 (TBS). 50 µl aliquots were taken from each tube for analysis at 1 hr, 1 day, 3 days, and 1 week using Thioflavin T fluorometry as described in example 2.

As shown in Figure 6, freshly suspended amylin alone following a 1-hour incubation at 37°C reached a maximum fluorescence of 1800 fluorescence units, which did not significantly change during the 1 week experimental period. The initial high fluorescence of amylin was attributed to amylin's ability to spontaneously form amyloid fibrils within a very short incubation period. Laminin at 100 nM did not significantly inhibit amylin fibril formation at all time points within the 1 week experimental period (Figure 6). In addition, no significant inhibition of amylin fibrillogenesis by laminin at decreasing concentrations (i.e. 40 nM , 15 nM and 5 nM) was observed, even though a decrease (but not significant) in amylin fibril formation was observed with 40 nM of laminin at 1 day, 3 days and 1 week (Figure 6). This study demonstrated that the inhibitory effects of laminin did not occur with amylin fibril formation, and demonstrated the specificity of the observed laminin inhibitory effects on Alzheimer's disease amyloid.

Example 5

Identification of V8 and Trypsin-Resistent Laminin Fragments which Interact with the Beta-Amyloid Protein of Alzheimer's Disease

In the next set of studies, we determined whether small fragment(s) of laminin generated by V8 or trypsin digestion would bind to Aß. This would enable one to determine the domain(s) of laminin which bind Aß and likely play a role in inhibition of Aß fibril formation and causing dissolution of preformed Alzheimer's amyloid fibrils (as

demonstrated in the invention).

For these experiments, Aß (1-40) was biotinylated according to the manufacturer's protocol (Pierce, Rockford, Illinois). For the ligand studies, intact EHS laminin was left undigested, or digested with V8 or trypsin (Sigma Chemical Company, St. Louis, MO, USA). More specifically, 2 µg of trypsin or V8 protease in 2 µl of 50 mM Tris-HCl buffer (pH 8.0) were added to 50 µl of laminin (50 µg)(in the same buffer) and incubated overnight at 37°C. The next day, 10 µl of protease-digested laminin (or undigested laminin) was mixed with 10 µl of 2X sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, and heated for 5 minutes in a boiling water bath. SDS-PAGE was performed according to the method of Laemmli (Laemmli, U.K. Nature 227:680-685, 1970), or according to the method of Schägger and Jagow (Schägger and Jagow, Anal. Biochem. 166:368-379, 1987) using a Mini-Protean II electrophoresis system (Biorad) with precast 4-15% Tris-Glycine or 10-20% tricine polyacrylamide gels, respectively, and under non-reducing conditions. Electrophoresis occurred at 200V for 45 minutes along with pre-stained molecular weight standards.

After SDS-PAGE (10-20% tricine or 4-15% Tris-Glycine gels) was performed as described above, the separated laminin and its fragments (total protein of 10 µg/lane) were transferred to polyvinylidine difluoride membrane (PVDF) using a Mini transblot electrophoresis transfer cell (Biorad, Hercules, CA, U.S.A.). Electrotransfer was performed at 100V for 2 hours. Following transfer, membranes were rinsed with methanol and dried. The fragment(s) of laminin involved in binding to Aß were then detected by using biotinylated-Aß (1-40), as described above. Blots were probed for 2 hours with 2 µM biotinylated Aß (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with strepavidin alkaline phosphatase conjugate

(Vectastain), rinsed again (as described above), and followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 7, V8-digested laminin produced multiple protein fragments which interacted with biotinylated Aß (1-40). Using a 4-15% Tris-Glycine gel system (Figure 7, lane 1), V8-resistent laminin fragments which interacted with Aß included fragments of ~400 kDa (which probably represented intact laminin which was left undigested), ~100-130 kDa, ~85 kDa, and a prominent fragment at ~ 55 kDa. Using a 10-20% tricine gel system (Figure 7, lane 2), V8-resistent laminin fragments which interacted with Aß included fragments of ~130 kDa, ~85 kDa, and a prominent fragment at ~ 55 kDa (Figure 7, lane 2, arrow). It is important to note that molecular size expressed in kilodaltons (kDa) are generally approximate. This study demonstrated that the smallest V8-resistent protein fragment of laminin which interacted with Aß (1-40) was ~55 kDa.

As shown in Figure 8, trypsin-digested laminin produced multiple protein fragments which interacted with biotinylated Aß (1-40). Using a 4-15% Tris-Glycine gel system (Figure 8, lane 1), trypsin-resistent laminin fragments which interacted with Aß included fragments of ~400 kDa (which probably represented intact laminin which was left undigested) , ~150-200 kDa, ~97 kDa, ~65 kDa and a prominent fragment at ~ 30 kDa. Using a 10-20% tricine gel system (Figure 8, lane 2), trypsin-resistent laminin fragments which interacted with Aß included fragments of ~97 kDa, ~90 kDa, ~65 kDa and a prominent fragment at ~ 30 kDa (Figure 8, lane 2, arrow). This study demonstrated that the smallest trypsin-resistent fragment of laminin which interacted with Aß (1-40) was ~30 kDa.

Example 6

Identification of Elastase-Resistent Laminin Fragments Which Interact with the Beta-Amyloid Protein of Alzheimer's Disease

In the next set of studies, we determined whether small fragment(s) of laminin generated by elastase digestion would bind to Aß. In addition , we sequenced and identified the region within elastase-resistent laminin which interacted with Aß. For these experiments, Aß (1-40) was biotinylated according to the manufacturer's protocol (Pierce, Rockford, Illinois). For the ligand studies, intact EHS laminin was left undigested, or digested with elastase (Sigma Chemical Company, St. Louis, MO, USA). For elastase digestion, 2 µg of elastase in 8 µl of 50 mM Tris-HCl buffer (pH 8.0) was added to 50 µl of laminin (50 µg)(in the same buffer) and incubated for 1.5 hours or 2.5 hours at 37°C. In addition, as a control, 2 µg of elastase in 50µl of 50 mM Tris-HCl buffer (pH 8.0) was incubated for 2.5 hours at 37°C. Following the appropriate incubation times as described above, 10 µl of each of the above incubations were mixed with 10 µl of 2X SDS-PAGE electrophoresis sample buffer, and heated for 5 minutes in a boiling water bath. SDS-PAGE was performed according to the method of Laemmli (Laemmli, Nature 227:680-685, 1970) using a Mini-Protean II electrophoresis system with precast 4-15% Tris-Glycine polyacrylamide gels, and under non-reducing conditions. Electrophoresis occurred at 200V for 45 minutes along with pre-stained molecular weight standards (Biorad).

After SDS-PAGE was performed as described above, the separated laminin fragments were transferred to PVDF using a Mini transblot electrophoresis transfer cell (Millipore, Bedford, MA, U.S.A.). Electrotransfer was performed at 100V for 2 hours.

Following transfer, membranes were rinsed with methanol, dried and cut into two equal parts which were used for Aß ligand blotting, or Coomassie blue staining and subsequent amino acid sequencing. The fragment(s) of laminin involved in binding to Aß were then detected by using biotinylated-Aß (1-40), as described above. Blots were probed for 2 hours with 2 µM biotinylated Aß (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with strepavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), and followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

For Coomassie blue staining, PVDF membranes were immersed with 0.2% Coomassie Brilliant blue (w/v) in 50 % methanol, 10% acetic acid, and 40% distilled water for 2 minutes, and then rinsed with 50% methanol, 10% acetic acid, and 40% distilled water until visible bands were observed, and no background staining was present. The 55 kDa Aß-binding laminin fragment, described below, was sent to the Biotechnology Service Center (Peptide Sequence Analysis Facility at the University of Toronto, Toronto, Ontario, Canada) and subjected to amino acid sequencing using a Porton 2090 Gas-Phase Microsequencer (Porton Instruments, Tarzana, CA) with on-line analysis of phenylthiohydantoin derivatives.

In Figure 9, Panel A represents an Aß ligand blot whereas Panel B represents the equivalent Coomassie blue stained blot. As shown in Figure 9 (Panel A, lanes 2 and 3), elastase-digested laminin produced multiple protein fragments which bound biotinylated Aß (1-40). Panel A, lane 1 represents undigested mouse EHS laminin, whereas lanes 2 and 3 represents laminin which had been digested with elastase for 1.5 hours or 2.5 hours,

respectively. Panel A, lane 4 represents elastase digestion for 2.5 hours in the absence of laminin. Undigested laminin (Fig. 9, Panel A, lane 1) which interacted with Aß included multiple bands from > ~400 kDa to >~86 kDa, with the most prominent Aß-interaction occurring with intact laminin (i.e. ~ 400 kDa). Elastase-resistent laminin protein fragments which interacted with Aß (Fig. 9, Panel A, lanes 2 and 3) included fragments of >~400kDa, ~130 kDa (arrowhead), ~80-90 kDa, ~65 kDa and a prominent band at ~ 55 kDa (arrow). The interaction of these elastase-resistent laminin protein fragments with Aß were only observed under non-reducing conditions suggesting that the Aß interaction was also conformation dependent. The 130kDa elastase resistent laminin fragment which interacts with Aß, is also believed to be part of the E8 fragment (see Figure 11), and is the same protein fragment of laminin that appears to be present in human serum and cerebrospinal fluid (see Examples 10 and 11). Figure 9, Panel A, lane 4 demonstrates that the band observed at ~29 kDa represents non-specific Aß binding due to the presence of the elastase enzyme alone.

Figure 9, Panel B demonstrates all of the multiple protein bands which were stained by Coomassie blue. Note, for example, in Panel B, lanes 2 and 3, that elastase digestion of laminin produced multiple protein fragments between ~55 kDa and ~90 kDa which did not bind Aß, and were not observed in the Aß ligand blot (Fig. 9, Panel A, lanes 2 and 3).

Example 7

An Aß-Binding Domain Within Laminin is Identified Within the Globular Repeats of the Laminin A Chain

The 55 kDa laminin fragment (ie. produced following 1.5 hours of elastase digestion) that demonstrated positive Aß binding interaction by ligand blotting was then

prepared (Fig. 9, Panel B, lane 2, arrow) in large amounts for amino acid sequencing (as described in example 6). Sequence data determined the exact location within laminin that was involved in binding to Aß. An 11-amino acid sequence was determined from sequencing of the 55 kDa band. The sequence identified was:

Leu-His-Arg-Glu-His-Gly-Glu-Leu-Pro-Pro-Glu (SEQ ID NO:1).

The specific Aß-binding domain within laminin was then identified by comparison to known mouse laminin sequence (Sasaki and Yamada, J. Biol. Chem. 262:17111-17117, 1987; Sasaki et al, Proc. Natl. Acad. Sci. 84:935-939, 1987; Durkin, et al, Biochem. 27:5198-5204, 1988; Sasaki et al, J. Biol. Chem. 263:16536-16544, 1988), since mouse EHS laminin was utilized in the studies of the present invention. In addition, the complete amino acid sequence within laminin was retrieved from the National Center for Biotechnology Information, Bethesda, Maryland, U.S.A.

Figure 10 shows the complete amino acid sequence of mouse laminin A chain (Genebank accession number P19137; SEQ ID NO: 4). The 11 amino acid protein fragment sequenced from the ~55 kDa protein within laminin which binds Aß is identified (Figure 10; bold underline and arrowhead; SEQ ID NO: 1) and matches exactly to the region within the third globular domain repeat of laminin A chain (Figure 11). The fourth globular domain repeat of mouse laminin A chain is shown as SEQ ID NO: 2 (Genebank Accession Number P19137; amino acids #2746-2922), whereas the fourth globular domain repeat of human laminin A chain is shown as SEQ ID NO: 3 (Genebank Accession Number P25391; amino acids #2737-2913).

Figure 11 shows two schematic representations of laminin (Colognato-Pyke et al, J. Biol. Chem. 270:9398-9406, 1995) and the newly discovered Aß-binding region of laminin

(shown in left panel; between the two arrowheads) which is situated within the last three globular domains of the laminin A chain. The left panel of figure 11 illustrates laminin and fragments generated following protease digestions. Elastase fragments E1', E1X (dark line border), E-alpha-35 and E4 all correspond to regions of the short arms of laminin. Long arm fragments are E8, E3 and cathepsin G fragment C8-9. The E8 fragment produced by elastase digestion of laminin contains the long arm fragments containing the distal part of the long arm and the G subdomains 1-3, and consists of a 130-150 kda (Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993).The E3 fragments also produced by elastase digestion of laminin contains the distal long arm globule with G subdomains 4 and 5. The E3 fragment shown in Figure 11, Panel A, has previously shown to be a doublet at ~60 kDa and ~55 kDa (Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993). This also confirms our discovery whereby the ~55 kDa fragment which we found to bind Aß is localized within the E3 region of laminin (Figure 11, Left Panel).

The right panel of Figure 11 depicts the function map with the alpha (A chain), ß (B1 chain) and gamma (B2 chain) chains of laminin shown in shades of decreasing darkness. EGF repeats are indicated by bars in the rod domains of the short arm. Domains, based on sequence analysis, are indicated in small Roman numerals and letters. The locations of heparin-binding, polymer-forming, and the active alpha1ß1 integrin-binding sites are shown in bold-face for the alpha-chain short arm. The long arm functions of heparin binding (heparin), alpha6ß1 integrin-recognition site (alpha6ß1), and dystroglycan (DG), mapped in other studies, are indicated in gray-shaded labels. It is interesting to note that the Aß-binding region of laminin is also a region involved in binding to heparin.

It should also be emphasized that the globular domain repeats of the laminin A chain likely interacts with Aß in a conformation dependent manner, since the interaction of the

~55-kilodalton elastase-resistent protein fragments with Aß was only observed under non-reducing conditions.

Example 8

Identification of Laminin and Laminin Protein Fragments in Human Serum and Cerebrospinal Fluid Derived from Alzheimer's disease, Type II Diabetes, and/or Normal Aged Patients

In the next study, western blotting techniques using a polyclonal antibody against laminin was used to determine whether intact laminin and/or laminin fragments were present in human serum and cerebrospinal fluid obtained from Alzheimer's disease, type II diabetes and/or normal aged patients. In this study, human serum was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations (where a score of 30 is normal, a score of 15 suggests moderate dementia and a score <10 suggests severe dementia), or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). In addition, human serum was obtained from the Diabetes Endocrinology Research Center at the University of Washington. The following human serums were obtained and analyzed as part of this study: 1) patient #9; a normal 67 yr old female with a mini-mental score of 30; 2) patient #5226 - a 70 year old female with confirmed moderate Alzheimer's disease who also had a mini-mental score of 12 ; 3) patient #5211- a 66 year old male with confirmed Alzheimer's disease who also had a mini-mental score of 25; 4) patient B- a 63 year old male who had confirmed type II diabetes; 5) patient #5223- a 68 year old female with confirmed Alzheimer's disease who also had a mini-mental score of 22; 6) patient #22- an 83 yr old normal aged female who also had a mini-mental score of 30;

7) patient #C- a 68 year old male with confirmed type II diabetes. Each of these serums were utilized in this study and represent lanes 1-7 (left side) of Figure 12 (in the same order as above).

In addition, cerebrospinal fluid was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations, or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). The following human cerebrospinal fluids were obtained as part of this study: 1) patient #6- a normal 64 year old female who had a mini-mental score of 30; 2) patient #7- a normal 67 year old male who had a mini-mental score of 30; 3) patient #8- a normal 80 year old female who had a mini-mental score of 30; 4) patient #9- a normal 67 year old female who had a mini-mental score of 30; 5) patient #1111P- a normal 78 year old female who had a mini-mental score of 30; 6) patient #50-a 66 year old male patient with probable moderate Alzheimer's disease as indicated by a mini-mental score of 15; 7) patient #54-a 73 year old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 8. Each of these cerebrospinal fluid samples were utilized in this study and represent lanes 1-7 (right side) of Figure 12 (in the same order as above).

For the study described above, 10 µl of human serum diluted at 1:10, or 10µl of undiluted human cerebrospinal fluid was added to 10 µl of SDS-PAGE buffer and ligand blots were prepared as in Example 6. Blots were probed for 2 hours with a polyclonal antibody (used at a dilution of 1:10,000 in TTBS) against EHS laminin (Sigma Chemical Company, St. Louis, MO). The membranes were then rinsed 3 times (10 seconds each) with TTBS and incubated for 1 hour with a biotinylated goat anti-rabbit IgG secondary

antibody diluted 1:1,000 with TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with strepavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 12, intact laminin (arrowheads) was present in human serum (lanes 1-7; left side) but not in human cerebrospinal fluid (lanes 1-7; right side). Qualitative observations suggest that intact laminin (as described above) may have been decreased in serum of Alzheimer's disease patients in comparison to controls (i.e. compare intact laminin in Figure 12, lane 1, left side-normal individual; to Figure 12, lane 2, left side-Alzheimer's disease patient). In addition to intact laminin, human serum derived from Alzheimer's disease, type II diabetes and normal aged patients also contained laminin immunoreactivity in a series of band from ~120 kDa to ~200 kDa (Figure 12, bands observed between the two arrows). On the other hand, cerebrospinal fluid samples did not contain intact laminin (Figure 12; lanes 1-7; right side) but only contained a series of laminin immunoreactive protein fragments from ~120 kDa to ~200 kDa (i.e. Figure 12, bands observed between the two arrows). This study determined that a series of laminin protein fragments are present in both human serum and cerebrospinal fluid of Alzheimer's disease, type II diabetes and normal aged patients, whereas intact laminin is only present in human serum. The novel discovery of the laminin fragments in human cerebrospinal fluid suggests that it may be used as a marker to determine the extent of laminin breakdown in the brain during Alzheimer's disease and other brain disorders.

Example 9

Identification of a ~130 Kilodalton Laminin Protein Fragment in Human Serum of Alzheimer's disease, Type II Diabetes and Normal Aged Patients which Binds Aß

In the next study, Aß ligand blotting techniques were utilized to identify whether laminin or laminin protein fragments present in human serum bind Aß. In this study, human serum was obtained from the Alzheimer's disease Research Center at the University of Washington from either living patients who may have had corresponding mini-mental state examinations (where a score of 30 is normal, a score of 15 suggests moderate dementia and a score <10 suggests severe dementia), or from living patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). In addition. human serum was obtained from the Diabetes Endocrinology Research Center at the University of Washington. The first six human serum samples (i.e. Figure 13, lanes 1-6) were the same serum samples as indicated in Example 8. In addition, Figure 13 lanes 7-10 consisted of human serum obtained from lane 7) patient #E- a 54 year old male with confirmed type II diabetes, lane 8) patient #5230- a 72 year old female with confirmed moderate Alzheimer's disease who had a mini-mental score of 19, lane 9) patient #E-a 54 year old male with confirmed type II diabetes, and lane 10) patient #F- a 69 year old male with confirmed type II diabetes.

For this study, Aß (1-40) was biotinylated according to the manufacturer's protocol (Pierce, Rockford, IL). For the ligand studies, following SDS-PAGE as described above in Example 8, separated laminin and its fragments present in human serum were transferred to polyvinylidine difluoride membrane (PVDF) using a Mini transblot electrophoresis transfer

cell. Electrotransfer was performed at 100V for 2 hours. Following transfer, membranes were rinsed with methanol and dried. The fragment(s) of laminin in human serum involved in binding to Aß were then detected by using biotinylated-Aß (1-40). Blots were probed for 2 hours with 1 µM biotinylated Aß (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with strepavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), and followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 13, Aß interacted with intact human laminin (arrow) in most samples of human serum. However, it was surprising to note that intact laminin was virtually absent in 2 of the 4 Alzheimer's disease patients serum (Fig. 13, lanes 5 and 8), suggesting that laminin-derived fragments may be important in Alzheimer's disease as a diagnostic marker. The most interesting discovery was that of all the laminin immunoreactive protein fragments found in human serum (i.e ~120 kDa to ~200 kDa, bands observed between the arrows, Figure 12, lanes 1-7, right side), only a prominent ~130 kDa band was found to interact with Aß (Figure 13, arrowhead). This same prominent band is approximately the same molecular weight of the E8 band generated from mouse laminin following elastase digestion (see Figure 9), and which also contains the globular domain repeats of the laminin A chain. This study therefore determined that besides intact laminin, human serum contains a ~130 kDa laminin fragment which binds to Aß, and may be important for keeping Aß soluble in biological fluids such as blood. This study also suggests that qualitative and quantitative assessment of laminin fragments in human serum may prove diagnostic for the extent and progression of Alzheimer's disease, type II diabetes and other amyloidoses.

Example 10

Identification of a ~130 Kilodalton Laminin Protein Fragment in Human Cerebrospinal Fluid of Alzheimer's disease and Normal Aged Patients which Binds Aß

In the next study, Aß ligand blotting techniques were utilized to identify whether laminin protein fragments (<200 kDa) present in human cerebrospinal fluid bind Aß. In this study, human cerebrospinal fluid was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations (where a score of 30 is normal, a score of 15 suggests moderate Alzheimer's disease and a score <10 suggests moderate Alzheimer's disease), or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). The following human cerebrospinal fluids were obtained and analyzed as part of this study (depicted in Figure 14, lanes 1-10): 1) patient #65- a 71 yr old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 0; 2) patient #54- a 73 yr old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 8.; 3) patient #6- a normal 64 yr old female who had a mini-mental score of 30; 4) patient #7- a normal 67 yr old male who had a mini-mental score of 30; 5) patient #8- a normal 80 yr old female who had a mini-mental score of 30; 6) patient #9- a normal 67 yr old female who had a mini-mental score of 30; 7) patient #1111P- a normal 78 yr old female who had a mini-mental score of 30; 8) patient #50-a 66 yr old male patient with probable moderate Alzheimer's disease as indicated by a mini-mental score of 15; 9) patient #52-a 69 yr old male with probable moderate Alzheimer's disease as indicated by a mini-mental score of 16; 10) patient #64-a 64 yr old male with probable severe Alzheimer's

disease as indicated by a mini-mental score of 0. Each of these cerebrospinal fluid samples were utilized in this study and represent lanes 1-10 of Figure 14 (in the same order as above).

For this study, Aß ligand blotting was employed as described in Example 9. The fragment(s) of laminin in human cerebrospinal fluid involved in binding to Aß were detected by using biotinylated-Aß (1-40). Blots were probed for 2 hours with 50 nM of biotinylated Aß (1-40) in TTBS. The rest of the Aß ligand blotting procedure is as described above in Example 9.

As shown in Figure 14, Aß interacted with laminin fragment bands between ~120 kDa and ~200 kDa in most samples of human cerebrospinal fluid. As observed in human serum, most samples of human cerebrospinal fluid also contained a prominent ~130 kDa laminin fragment (Figure 14, arrow) which interacted with Aß. No intact Aß-binding laminin was found in human cerebrospinal fluid (not shown), as previously demonstrated (Figure 12, Example 8). Again, this same prominent ~130 kDa Aß-binding laminin fragment present in human cerebrospinal fluid is approximately the same molecular weight of the E8 band generated from laminin, and which also contains the globular domain repeats of the laminin A chain. This study therefore determined that human cerebrospinal fluid also contains a ~130 kDa laminin fragment which binds to Aß, and may be important for keeping Aß soluble in biological fluids such as cerebrospinal fluid.

Further Aspects and Utilizations of the Invention

Laminin-Derived Protein Fragments and Polypeptides

One therapeutic application of the present invention is to use laminin, laminin protein fragments which bind Aß or other amyloid proteins, and/or laminin polypeptides derived from amino acid sequencing of the laminin fragments which bind Aß (such as the ~130 kilodalton protein described herein) or other amyloid proteins, as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease and Down's syndrome (wherein the specific amyloid is referred to as beta-amyloid protein or Aß), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (wherein the specific amyloid is referred to as AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell dyscrasias (wherein the specific amyloid is referred to as AL amyloid), the amyloid associated with type II diabetes (wherein the specific amyloid is referred to as amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (wherein the specific amyloid is referred to as PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (wherein the specific amyloid is referred to as $beta_2$-microglobulin amyloid), the amyloid associated with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (wherein the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (wherein the specific amyloid is referred to as variants of procalcitonin).

The polypeptides referred to above may be a natural polypeptide, a synthetic polypeptide or a recombinant polypeptide. The fragments, derivatives or analogs of the polypeptides to any laminin fragment referred to herein may be a) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue and such substituted amino acid residue may or may not be encoded by the genetic code, or b) one in which one or more of the amino acid residues includes a substituent group, or c) one in which the mature polypeptide is fused with another compound, such as a compound used to increase the half-life of the polypeptide (for example, polylysine), or d) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of the invention.

The tertiary structure of proteins refers to the overall 3-dimensional architecture of a polypeptide chain. The complexity of 3-dimensional structure arises from the intrinsic ability of single covalent bonds to be rotated. Rotation about several such bonds in a linear molecule will produce different nonsuperimpossable 3-dimensional arrangements of the atoms that are generally described as conformations.

Protein conformation is an essential component of protein-protein, protein-substrate, protein-agonist, protein-antagonist interactions. Changes in the component amino acids of protein sequences can result in changes that have little or no effect on the resultant protein conformation. Conversely, changes in the peptide sequences can have effects on the protein conformation resulting in reduced or increased protein-protein, etc. interactions. Such changes and their effects are generally disclosed in Proteins: Structures and Molecular Properties by Thomas Creightonm W.H. Freeman and Company, New York, 1984 which

is hereby incorporated by reference.

"Conformation" and "conformation similarity" when used in this specification and claims refers to a polypeptide's ability (or any other organic or inorganic molecule) to assume a given shape, through folding and the like, so that the shape, or conformation, of the molecule becomes an essential part of it's functionality, sometimes to the exclusion of its chemical makeup. It is generally known that in biological processes two conformational similar molecules may be interchangeable in the process, even the chemically different. "Conformational similarity" refers to the latter interchangeability or substitutability. For example, laminin and laminin-derived protein fragments are among the subjects of the invention because they have been shown to bind the Aß protein and render it inactive in fibril formation; it is contemplated that other molecules that are conformationally similar to laminin, or any claimed laminin fragment or polypeptide, may be substituted in the claimed method to similarly render the Aß inactive in fibrillogenesis and other amyloid processes. In general it is contemplated that levels of conformational similarity at or above 70% are sufficient to assume homologous functionality in the claimed processes, though reduced levels of conformational similarity may be made to serve as well. Conformational similar levels at or above 90% should provide some level of additional homologue functionality.

Thus, one skilled in the art would envisage that changes can be made to the laminin sequence, or fragments or polypeptides thereof, that would increase, decrease or have no effect on the binding of laminin or fragments thereof, to Aß amyloid. In addition, one skilled in the art would envisage various post-translational modifications such as phosphorylation, glycosylation and the like would alter the binding of laminin, laminin fragments or laminin polypeptides to Aß amyloid.

The polypeptides of the present invention include the polypeptides or fragments of laminin described herein, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

Fragments or portions of the polypeptides or fragments of laminin of the present invention may be employed for producing the corresponding full-length polypeptides by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full length polypeptides.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Chemical polypeptide synthesis is a rapidly evolving area in the art, and methods of solid phase polypeptide synthesis are well-described in the following references, hereby entirely incorporated by reference (Merrifield, J. Amer. Chem. Soc. 85:2149-2154, 1963; Merrifield, Science 232:341-347, 1986; Fields, Int. J. Polypeptide Prot. Res. 35, 161, 1990).

Recombinant production of laminin polypeptides can be accomplished according to known method steps. Standard reference works seting forth the general principles of recombinant DNA technology include Watson, Molecular Biology of the Gene, Volumes I and II, The Benjamin/Cummings Publishing Company Inc., publisher, Menlo Park, Calif. 1987; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, publisher, New York, N.Y. 1987; 1992; and Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, publisher, Cold Spring Harbor, N.Y. 1989, the entire contents of which references are herein incorporated by reference.

The polypeptides of the present invention may also be utilized as research reagents and materials for discovery of treatments and diagnostics for human diseases.

Antibodies

Antibodies generated against the polypeptides corresponding to specific sequences recognizing the laminin fragments of the present invention which bind Aß or other amyloid proteins can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptides from tissue expressing that polypeptide. Preferred embodiments include, but are not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and

fragments thereof, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies, chimeric antibodies, anti-idiotypic antibodies to antibodies specific for laminin-derived protein fragments or polypeptides of the present invention.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, Nature 256:495-497, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al, Immunology Today 4:72, 1983), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp.77-96, 1985). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, which are primarily used to reduce immunogenicity in application and to increase yields in production. Chimeric

antibodies and methods for their production are known in the art (ex. Cabilly et al, Proc. Natl. Acad. Sci. U.S.A 81:3273-3277, 1984; Harlow and Lane: Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988).

An anti-idiotypic antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An anti-iodiotypic antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-iodiotypic antibody is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-idiotypic antibody). See, for example, U.S. Patent No. 4,699,880, which is herein incorporated by reference.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al, J. Nucl. Med. 24:316-325, 1983).

The antibodies or fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect laminin or laminin-derived fragments in a sample or to detect presence of cells which express a laminin polypeptide of the present invention.This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric or fluorometric detection.

One of the ways in which a laminin fragment antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric, or by visual means. Enzymes which can be used detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colometric methods which employ a chromogenic substrate for the enzyme. Detection can be accomplished by colometric methods which employ a chromogenic substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate with similarly prepared standards (see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, N.Y. 1987, 1992).

Detection may be accomplished using any of a variety of other immunoassays. For example, by radiolabeling of the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work et al, North Holland Publishing Company, NY (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, incorporated entirely by reference herein. The radioactive isotope can be detected by such means as the use of a gamma-counter, a scintillation counter or by autoradiography.

It is also possible to label a laminin fragment polypeptide antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine, commercially available, e.g., from Molecular Probes, Inc. (Eugene, Oregon, U.S.A.).

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}EU, or other of the lanthanide series. These metals can be attached to the antibody using such metal groups as diethylenetriamine pentaacetic acid (EDTA).

The antibody can also be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction, Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt, and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

The antibodies (or fragments thereof) useful in the present invention may be

employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of a laminin fragment of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of a laminin fragment polypeptide but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

In accordance with yet a further aspect of the present invention there are provided antibodies against laminin, laminin fragments and/or laminin-derived polypeptides which interact with Aß or other amyloid proteins, or derivatives thereof. These antibodies can be used for a number of important diagnostic and/or therapeutic applications as described herein. In one aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin, laminin fragments and/or laminin-derived polypeptides which bind Aß or other amyloid proteins, may be utilized for Western blot analysis (using standard Western blotting techniques knowledgeable to those skilled in the art) to detect the presence of amyloid protein-binding laminin fragments or amyloid protein-binding laminin polypeptides in human tissues and in tissues of other species. Western blot analysis can also be used to determine the apparent size of each amyloid protein-binding laminin fragment. In addition, Western blotting following by scanning densitometry (known to those skilled in the art) can be used to quantitate and compare levels of each of the laminin fragments in tissue samples, biological fluids or biopsies obtained from individuals with specific diseases (such as the amyloid diseases) in comparison to tissue samples, biological fluids or biopsies obtained

from normal individuals or controls. Biological fluids, include, but are not limited to, blood, plasma, serum, cerebrospinal fluid, sputum, saliva, urine and stool.

In yet another aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin, laminin fragments and/or laminin-derived peptides which bind Aß or other amyloid proteins, can be utilized for immunoprecipitation studies (using standard immunoprecipitation techniques known to one skilled in the art) to detect laminin, laminin fragments and/or laminin-derived peptides which bind Aß or other amyloid proteins, in tissues, cells and/or biological fluids. Use of the laminin, laminin fragment and/or laminin-derived peptide antibodies for immunoprecipitation studies can also be quantitated to determine relative levels of laminin, laminin fragments and/or laminin-derived peptides which interact with Aß or other amyloid proteins, in tissues, cells and/or biological fluids. Quantitative immunoprecipitation can be used to compare levels of laminin, laminin fragments and/or laminin amyloid protein-binding peptides in tissue samples, biological fluids or biopsies obtained from individuals with specific diseases (such as the amyloid diseases) in comparison to tissue samples, biological fluids or biopsies obtained from normal individuals or controls.

Therapeutic Applications

Yet another aspect of the present invention is to make use of laminin, laminin fragments and/or laminin-derived polypeptides as amyloid inhibitory therapeutic agents. The laminin-derived peptide sequences or fragments can be synthesized utilizing standard techniques (ie. using an automated synthesizer). Laminin, laminin fragments and/or laminin-derived polypeptides which bind Aß or other amyloid proteins, can be used as potential blocking therapeutics for the interaction of laminin in a number of biological processes and

diseases (such as in the amyloid diseases described above). In a preferred embodiment, specific peptides made against the amino acid sequence of laminin contained within the ~55 kDa laminin fragment (i.e. globular repeats within the laminin A chain; SEQ ID NO 3) described in the present invention, may be used to aid in the inhibition of amyloid formation, deposition, accumulation, and /or persistence in a given patient. Likewise, in another preferred embodiment anti-idiotypic antibodies made against laminin, laminin fragments and/or laminin-derived polypeptides (as described above) may be given to a human patient as potential blocking antibodies to disrupt continued amyloid formation, deposition, accumulation and/or persistence in the given patient.

Preparations of laminin-derived polypeptides for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain axillary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets, pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, can be prepared according to routine methods and are known in the art.

In yet another aspect of the invention, laminin, laminin fragments and/or laminin-derived polypeptides may be used as an effective therapy to block amyloid formation, deposition, accumulation and/or persistence as observed in the amyloid diseases. For example, the invention includes a pharmaceutical composition for use in the treatment of amyloidoses comprising a pharmaceutically effective amount of a laminin, laminin fragment and/or laminin-derived polypeptide anti-idiotypic antibody and a pharmaceutically acceptable carrier. The compositions may contain the laminin, laminin fragments and/or laminin-derived polypeptide anti-idiotypic antibody, either unmodified, conjugated to a potentially

therapeutic compound, conjugated to a second protein or protein portion or in a recombinant form (ie. chimeric or bispecific laminin, laminin fragment and/or laminin polypeptide antibody). The compositions may additionally include other antibodies or conjugates. The antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, topical, intravenous, intra-arterial, intraperitoneal, oral, intralymphatic, intramuscular or intralumbar. Intravenous administration is preferred. The compositions of the invention can be a variety of dosage forms, with the preferred form depending upon the mode of administration and the therapeutic application. Optimal dosage and modes of administration for an individual patient can readily be determined by conventional protocols.

Laminin, laminin-derived protein fragments, and laminin-derived polypeptides, or antibodies of the present invention may be administered by any means that achieve their intended purpose, for example, to treat laminin involved pathologies, such as Alzheimer's disease and other amyloid diseases, or other related pathologies, using a laminin-derived polypeptide described herein, in the form of a pharmaceutical composition.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal or buccal routes. Alternatively, or concurrently, administration may be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

A preferred mode of using a laminin-derived polypeptide, or antibody pharmaceutical composition of the present invention is by oral administration or intravenous application.

A typical regimen for preventing, suppressing or treating laminin-involved pathologies, such as Alzheimer's disease amyloidosis, comprises administration of an effective amount of laminin-derived polypeptides, administered over a period of one or several days, up to and including between one week and about 24 months.

It is understood that the dosage of the laminin-derived polypeptides of the present invention administered in vivo or in vitro will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation.

The total dose required for each treatment may be administered by multiple doses or in a single dose. A laminin-derived polypeptide may be administered alone or in conjunction with other therapeutics directed to laminin-involved pathologies, such as Alzheimer's disease or amyloid diseases, as described herein.

Effective amounts of a laminin-derived polypeptide or composition, which may also include a laminin-fragment derived antibody, are about 0.01μg to about 100mg/kg body weight, and preferably from about 10 μg to about 50 mg/kg body weight, such as 0.05, 0.07, 0.09, 0.1, 0.5, 0.7, 0.9., 1, 2, 5, 10, 20, 25, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg/kg.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain axillary agents or excipients

which are known in the art. Pharmaceutical compositions comprising at least one laminin-derived polypeptide, such as 1-10 or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 laminin-derived polypeptides, of the present invention may include all compositions wherein the laminin-derived polypeptide is contained in an amount effective to achieve its intended purpose. In addition to at least one laminin-derived polypeptide, a pharmaceutical composition may contain suitable pharmaceutically acceptable carriers, such as excipients, carriers and/or axillaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions comprising at least one laminin-derived polypeptide or antibody may also include suitable solutions for administration intravenously, subcutaneously, dermally, orally, mucosally, rectally or may by injection or orally, and contain from about 0.01 to 99 percent, preferably about 20 to 75 percent of active component (i.e. polypeptide or antibody) together with the excipient. Pharmaceutical compositions for oral administration include pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, and syrups.

The laminin, laminin-derived protein fragments, and laminin-derived polypeptides for Alzheimer's disease and other central nervous system amyloidoses may be optimized to cross the blood-brain barrier. Methods of introductions include but are not limited to systemic administration, parenteral administration i.e., via an intraperitoneal, intravenous, perioral, subcutaneous, intramuscular, intraarterial, intradermal, intramuscular, intranasal, epidural and oral routes. In a preferred embodiment, laminin, laminin-derived protein fragments, and laminin-derived polypeptides may be directly administered to the cerebrospinal fluid by intraventricular injection. In a specific embodiment, it may be

desirable to administer laminin, laminin-derived protein fragments, and laminin-derived polypeptides locally to the area or tissue in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, by injection, by infusion using a cannulae with osmotic pump, by means of a catheter, by means of a suppository, or by means of an implant.

In yet another embodiment laminin, laminin-derived protein fragments, and laminin-derived polypeptides may be delivered in a controlled release system, such as an osmotic pump. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, ie. the brain, thus requiring only a fraction of the systemic dose.

In yet another aspect of the present invention, peptidomimetic compounds modelled from laminin, laminin fragments and/or laminin-derived polypeptides identified as binding Aß or other amyloid proteins, may serve as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. Peptidomimetic modelling is implemented by standard procedures known to those skilled in the art.

In yet another aspect of the present invention, compounds that mimic the 3-dimensional Aß binding site on laminin using computer modelling, may serve as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. Design and production of such compounds using computer modelling technologies is implemented by standard procedures known to those skilled in the art.

Recombinant DNA technology, including human gene therapy, has direct

applicability to the laminin proteins and their fragments, of this invention. One skilled in the art can take the peptide sequences disclosed herein and create corresponding nucleotide sequences that code for the corresponding peptide sequences. These sequences can be cloned into vectors such as retroviral vectors, and the like. These vectors can, in turn, be transfected into human cells such as hepatocytes or fibroblasts, and the like. Such transfected cells can be introduced into humans to treat amyloid diseases. Alternatively, the genes can be introduced into the patients directly. The basic techniques of recombinant DNA technology are known to those of ordinary skill in the art and are disclosed in Recombinant DNA Second Edition, Watson, et al., W.H. Freeman and Company, New York, 1992, which is hereby incorporated by reference.

Diagnostic Applications

Another aspect of the invention is to provide polyclonal and/or monoclonal antibodies against laminin, laminin fragments and/or laminin-derived polypeptides which bind Aß or other amyloid proteins, which would be utilized to specifically detect laminin, laminin fragments and/or laminin-derived peptides in human tissues and/or biological fluids. In one preferred embodiment, polyclonal or monoclonal antibodies made against a peptide portion or fragment of laminin, can be used to detect and quantify laminin, laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. Polyclonal and/or monoclonal peptide antibodies can also be utilized to specifically detect laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. In a preferred embodiment, a polyclonal or monoclonal antibody made specifically against a peptide portion or fragment of ~55 kDa elastase-resistent protein which binds Aß (as described herein), can be used to detect and quantify this laminin fragment in human tissues and/or biological fluids. In another preferred embodiment, a polyclonal or

monoclonal antibody made specifically against a peptide portion or fragment of ~130 kDa laminin-derived protein which is present in human biological fluids and binds Aß (as described herein), can be used to detect and quantify this laminin fragment in human tissues and/or biological fluids. Other preferred embodiments include, but are not limited to, making polyclonal or monoclonal antibodies made specifically against a peptide portion or fragment of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

For detection of laminin fragments and/or laminin-derived polypeptides described above in human tissues, cells, and/or in cell culture, the polyclonal and/or monoclonal antibodies can be utilized using standard immunohistochemical and immunocytochemical techniques, known to one skilled in the art.

For detection and quantitation of laminin, laminin fragments and/or laminin-derived polypeptides in biological fluids, including cerebrospinal fluid, blood, plasma, serum, urine, sputum, and/or stool, various types of ELISA assays can be utilized, known to one skilled in the art. An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier, and a quantity of detectable labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

In a preferred embodiment, a "sandwich" type of ELISA can be used. Using this preferred method a pilot study is first implemented to determine the quantity of binding of each laminin-fragment monoclonal antibody to microtiter wells. Once this is determined, aliquots (usually in 40 µl of TBS; pH 7.4) of the specific laminin-fragment antibody are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C. A series of blank wells not containing any laminin-fragment specific monoclonal antibody are also utilized as controls. The next day, non-bound monoclonal antibody is shaken off the microtiter wells. All of the microtiter wells (including the blank wells) are then blocked by incubating for 2 hours with 300 µl of Tris-buffered saline containing 0.05% Tween-20 (TTBS) plus 2% bovine serum albumin, followed by 5 rinses with TTBS. 200 µl of cerebrospinal fluid, blood, plasma, serum, urine, sputum, and/or stool and/or any other type of biological sample is then diluted (to be determined empirically) in TTBS containing 2% bovine serum albumin and placed in wells (in triplicate) containing bound laminin-fragment antibody (or blank) and incubated for 2 hours at room temperature. The wells are then washed 5 times with TTBS. A second biotinylated-monoclonal antibody against the same laminin-derived fragment (but which is against a different epitope) is then added to each well (usually in 40 µl of TBS; pH 7.4) and allowed to bind for 2 hours at room temperature to any laminin-fragment captured by the first antibody. Following incubation, the wells are washed 5 times with TTBS. Bound materials are then detected by incubating with 100 µl of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% BSA) for 1 hour on a rotary shaker. After 5 washes with TTBS, a substrate solution (100 µl, OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes). The reaction is stopped with 50 µl of 4N sulfuric acid and read on a standard spectrophotometer at 490 nm. This ELISA can be utilized to determine differences in specific laminin fragments (and/or Aß-binding laminin fragments) in biological fluids which can serve as a diagnostic marker to follow the

progression on a live patient during the progression of disease (ie. monitoring of amyloid disease as an example). In addition, quantitative changes in laminin fragments can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets a given amyloid disease such as Alzheimer's disease. Such assays can be provided in a kit form.

A competition assay may also be employed wherein antibodies specific to laminin, laminin fragments and/or laminin-derived polypeptides are attached to a solid support and labelled laminin, laminin fragments and/or laminin-derived polypeptides and a sample derived from a host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to the quantity of laminin, laminin fragments and/or laminin-derived polypeptides in the sample. This standard technique is known to one skilled in the art.

Another object of the present invention is to use laminin, laminin fragments and/or laminin-derived polypeptides, in conjunction with laminin, laminin fragment and/or laminin-derived peptide antibodies, in an ELISA assay to detect potential laminin, laminin fragment and/or laminin-derived peptide autoantibodies in human biological fluids. Such a diagnostic assay may be produced in a kit form. In a preferred embodiment, peptides containing the sequences of laminin, laminin-derived fragments and laminin-derived polypeptides as in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above, will be used to initially bind to microtiter wells in an ELISA plate. A pilot study is first implemented to determine the quantity of binding of each laminin fragment

polypeptide to microtiter wells. Once this is determined, aliquots (usually 1-2μg in 40 μl of TBS; pH 7.4) of specific laminin fragment polypeptides (as described herein) are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C. All the microtiter wells (including blank wells without the laminin fragment polypeptides) are blocked by incubating for 2 hours with 300 μl of Tris-buffered saline (pH 7.4) with 0.05% Tween-20 (TTBS), containing 2% albumin. This is followed by 5 rinses with TTBS. The patients' biological fluids (i.e., cerebrospinal fluid, blood, plasma, serum, sputum, urine, and/or stool) are then utilized and 200 μl are diluted (to be determined empirically) with TTBS containing 2% bovine serum albumin, and placed in microtiter wells (in triplicate) containing a specific laminin fragment polypeptide or blank wells (which do not contain peptide), and are incubated at 1.5 hours at room temperature. Any autoantibodies present in the biological fluids against the laminin fragment will bind to the substrate bound laminin fragment polypeptide (or fragments thereof). The wells are then rinsed by washing 5 times with TTBS. 100 μl of biotinylated polyclonal goat anti-human IgGs (Sigma Chemical company, St. Louis, MO, USA), diluted 1:500 in TTBS with 0.1% bovine serum albumin, is then aliquoted into each well. Bound materials are detected by incubating with 100 μl of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% bovine serum albumin) for 1 hour on a rotary shaker. Following 5 washes with TTBS, substrate solution (100 μl, OPD-Sigma Fast from Sigma Chemical Company, St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes). The reaction is stopped with 50 μl of 4N sulfuric acid added to each well and read on a standard spectrophotometer at 490 nm. This assay system can be utilized to not only detect the presence of autoantibodies against laminin fragments in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin fragment autoantibody levels. It is believed that patients demonstrating excessive laminin fragment formation, deposition, accumulation and/or persistence as may be observed in the amyloid diseases, will also carry autoantibodies

against the laminin fragments in their biological fluids. Various ELISA assay systems, knowledgeable to those skilled in the art, can be used to accurately monitor the degree of laminin fragments in biological fluids as a potential diagnostic indicator and prognostic marker for patients during the progression of disease (ie. monitoring of an amyloid disease for example). Such assays can be provided in a kit form. In addition, quantitative changes in laminin fragment autoantibody levels can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets a given amyloid disease.

Other diagnostic methods utilizing the invention include diagnostic assays for measuring altered levels of laminin, laminin fragments and/or laminin-derived polypeptides in various tissues compared to normal control tissue samples. Assays used to detect levels of laminin, laminin fragments and/or laminin-derived polypeptides in a sample derived from a host are well-known to those skilled in the art and included radioimmunoassays, competitive-binding assays, Western blot analysis and preferably ELISA assays (as described above).

Yet another aspect of the present invention is to use the antibodies recognizing laminin, laminin fragments and/or laminin-derived polypeptides for labellings, for example, with a radionucleotide, for radioimaging or radioguided surgery, for in vivo diagnosis, and/or for in vitro diagnosis. In one preferred embodiment, radiolabelled peptides or antibodies made (by one skilled in the art) against laminin, laminin fragments and/or laminin-derived polypeptides may be used as minimally invasive techniques to locate laminin, laminin fragments and/or laminin-derived polypeptides, and concurrent amyloid deposits in a living patient. These same imaging techniques could then be used at regular intervals (ie. every 6 months) to monitor the progression of the amyloid disease by following the specific levels of laminin, laminin fragments and/or laminin-derived

polypeptides.

Yet another aspect of the present invention is to provide a method which can evaluate a compound's ability to alter (diminish or eliminate) the affinity of a given amyloid protein (as described herein) or amyloid precursor protein, to laminin, laminin-derived fragments or laminin-derived polypeptides. By providing a method of identifying compounds which affect the binding of amyloid proteins, or amyloid precursor proteins to such laminin-derived fragments, the present invention is also useful in identifying compounds which can prevent or impair such binding interaction. Thus, compounds can be identified which specifically affect an event linked with the amyloid formation, amyloid deposition, and/or amyloid persistence condition associated with Alzheimer's disease and other amyloid diseases as described herein.

According to one aspect of the invention, to identify for compounds which allow the interaction of amyloid proteins or precursor proteins to laminin-derived fragments or laminin polypeptides, either amyloid or laminin fragments are immobilized, and the other of the two is maintained as a free entity. The free entity is contacted with the immobilized entity in the presence of a test compound for a period of time sufficient to allow binding of the free entity to the immobilized entity, after which the unbound free entity is removed. Using antibodies which recognize the free entity, or other means to detect the presence of bound components, the amount of free entity bound to immobilized entity can be measured. By performing this assay in the presence of a series of known concentrations of test compound and, as a control, the complete absence of test compound, the effectiveness of the test compound to allow binding of free entity to immobilized entity can be determined and a quantitative determination of the effect of the test compound on the affinity of free entity to immobilized entity can be made. By comparing the binding affinity of the amyloid-laminin fragment

complex in the presence of a test compound to the binding affinity of the amyloid-laminin fragment complex in the absence of a test compound, the ability of the test compound to modulate the binding can be determined.

In the case in which the amyloid is immobilized, it is contacted with free laminin-derived fragments or polypeptides, in the presence of a series of concentrations of test compound. As a control, immobilized amyloid is contacted with free laminin-derived polypeptides, or fragments thereof in the absence of the test compound. Using a series of concentrations of laminin-derived polypeptides, the dissociation constant (K_d) or other indicators of binding affinity of amyloid-laminin fragment binding can be determined. In the assay, after the laminin-derived polypeptides or fragments thereof is placed in contact with the immobilized amyloid for a sufficient time to allow binding, the unbound laminin polypeptides are removed. Subsequently, the level of laminin fragment-amyloid binding can be observed. One method uses laminin-derived fragment antibodies, as described in the invention, to detect the amount of specific laminin fragments bound to the amyloid or the amount of free laminin fragments remaining in solution.This information is used to determine first qualitatively whether or not the test compound can allow continued binding between laminin-derived fragments and amyloid. Secondly, the data collected from assays performed using a series of test compounds at various concentrations, can be used to measure quantitatively the binding affinity of the laminin fragment-amyloid complex and thereby determine the effect of the test compound on the affinity between laminin fragments and amyloid.Using this information, compounds can be identified which do not modulate the binding of specific laminin fragments to amyloid and thereby allow the laminin-fragments to reduce the amyloid formation, deposition, accumulation and/or persistence, and the subsequent development and persistence of amyloidosis.

Therefore a kit for practicing a method for identifying compounds useful which do not alter laminin, laminin-derived fragments or laminin-derived polypeptides to an immobilized amyloid protein, said kit comprising a) a first container having amyloid protein immobilized upon the inner surface, b) a second container which contains laminin, laminin-derived fragments or laminin-derived polypeptides dissolved in solution, c) a third container which contains antibodies specific for said laminin, laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution, and d) a fourth container which contains labelled antibodies specific for laminin, laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: Gerardo Castillo and Alan Snow
(ii) TITLE OF INVENTION: Therapeutic and Diagnostic Applications of Laminin and Laminin-Derived Protein Fragments
(iii) NUMBER OF SEQUENCES: 11
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Patrick M. Dwyer
(B) STREET: 1919 One Union Square, 600 University Street
(C) CITY: Seattle
(D) STATE: WA (Washington)
(E) COUNTRY: United States of America
(F) ZIP: 98101
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette - 3.50 inch, 1.44 Mb storage
(B) COMPUTER: IBM PC
(C) OPERATING SYSTEM: PC-DOS (Windows NT Version 4.0, '95)
(D) SOFTWARE: WordPerfect 5.2
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/947,057
(B) FILING DATE: 08-October-1997
(C) CLASSIFICATION: U.S. Utility Appl.
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/027,981
(B) FILING DATE: 08-October-1996
(C) CLASSIFICATION: U.S. Provisional Appl.
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Dwyer, Patrick M.
(B) REGISTRATION NUMBER: 32,411
(C) REFERENCE/DOCKET NUMBER: PROTEO.P03
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 343-7074
(B) TELEFAX: (206) 343-7085

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 11 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P19137

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Leu His Arg Glu His Gly Glu Leu Pro Pro Glu
1 5 10

INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 177 AMINO ACIDS
(B) TYPE: AMINO ACID

(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P19137 (AMINO ACIDS #2746-2922)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Leu Gln Val Gln Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu Ile Tyr Tyr Val Ala
1               5                  10                  15                  20
His Gln Asn Gln Met Asp Tyr Ala Thr Leu Gln Leu Gln Glu Gly Arg Leu His Phe Met
            25                  30                  35                  40
Phe Asp Leu Gly Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu Leu Ser Asp Gly Lys
        45                  50                  55                  60
Trp His Thr Val Lys Thr Glu Tyr Ile Lys Arg Lys Ala Phe Met Thr Val Asp Gly Gln
    65                  70                  75                  80
Glu Ser Pro Ser Val Thr Val Val Gly Asn Ala Thr Thr Leu Asp Val Glu Arg Lys Leu
                85                  90                  95                  100
Tyr Leu Gly Gly Leu Pro Ser His Tyr Arg Ala Arg Asn Ile Gly Thr Ile Thr His Ser
            105                 110                 115                 120
Ile Pro Ala Cys Ile Gly Glu Ile Met Val Asn Gly Gln Gln Leu Asp Lys Asp Arg Pro
        125                 130                 135                 140
Leu Ser Ala Ser Ala Val Asp Arg Cys Tyr Val Val Ala Gln Glu Gly Thr Phe Phe Glu
    145                 150                 155                 160
Gly Ser Gly Tyr Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Arg Leu Asp
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P25391 (AMINO ACIDS #2737-2913)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Leu Ser Val Glu Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu Ile Tyr Tyr Met Ala
1               5                  10                  15                  20
His Gln Asn Gln Ala Asp Tyr Ala Val Leu Gln Leu His Gly Gly Arg Leu His Phe Met
            25                  30                  35                  40
Phe Asp Leu Gly Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu Leu Ser Asp Gly Lys
        45                  50                  55                  60
Trp His Thr Val Lys Thr Asp Tyr Val Lys Arg Lys Gly Phe Ile Thr Val Asp Gly Arg
    65                  70                  75                  80
Glu Ser Pro Met Val Thr Val Val Gly Asp Gly Thr Met Leu Asp Val Glu Gly Leu Phe
                85                  90                  95                  100
Tyr Leu Gly Gly Leu Pro Ser Gln Tyr Gln Ala Arg Lys Ile Gly Asn Ile Thr His Ser
            105                 110                 115                 120
Ile Pro Ala Cys Ile Gly Asp Val Thr Val Asn Ser Lys Gln Leu Asp Lys Asp Ser Pro
        125                 130                 135                 140
Val Ser Ala Phe Thr Val Asn Arg Cys Tyr Ala Val Ala Gln Glu Gly Thr Tyr Phe Asp
```

```
                145                 150                 155                 160
Gly Ser Gly Tyr Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Gln Ser Asp
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3084 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P19137

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Arg Gly Ser Gly Thr Gly Ala Ala Leu Leu Val Leu Leu Ala Ser Val Leu Trp Val
1               5                  10                  15                  20
Thr Val Arg Ser Gln Gln Arg Gly Leu Phe Pro Ala Ile Leu Asn Leu Ala Thr Asn Ala
                25                  30                  35                  40
His Ile Ser Ala Asn Ala Thr Cys Gly Glu Lys Gly Pro Glu Met Phe Cys Lys Leu Val
                45                  50                  55                  60
Glu His Val Pro Gly Arg Pro Val Arg His Ala Gln Cys Arg Val Cys Asp Gly Asn Ser
                65                  70                  75                  80
Thr Asn Pro Arg Glu Arg His Pro Ile Ser His Ala Ile Asp Gly Thr Asn Asn Trp Trp
                85                  90                  95                  100
Gln Ser Pro Ser Ile Gln Asn Gly Arg Glu Tyr His Trp Val Thr Val Thr Leu Asp Leu
                105                 110                 115                 120
Arg Gln Val Phe Gln Val Ala Tyr Ile Ile Ile Lys Ala Ala Asn Ala Pro Arg Pro Gly
                125                 130                 135                 140
Asn Trp Ile Leu Glu Arg Ser Val Asp Gly Val Lys Phe Lys Pro Trp Gln Tyr Tyr Ala
                145                 150                 155                 160
Val Ser Asp Thr Glu Cys Leu Thr Arg Tyr Lys Ile Thr Pro Arg Arg Gly Pro Pro Thr
                165                 170                 175                 180
Tyr Arg Ala Asp Asn Glu Val Ile Cys Thr Ser Tyr Tyr Ser Lys Leu Val Pro Leu Glu
                185                 190                 195                 200
His Gly Glu Ile His Thr Ser Leu Ile Asn Gly Arg Pro Ser Ala Asp Asp Pro Ser Pro
                205                 210                 215                 220
Gln Leu Leu Glu Phe Thr Ser Ala Arg Tyr Ile Arg Leu Arg Leu Gln Arg Ile Arg Thr
                225                 230                 235                 240
Leu Asn Ala Asp Leu Met Thr Leu Ser His Arg Asp Leu Arg Asp Leu Asp Pro Ile Val
                245                 250                 255                 260
Thr Arg Arg Tyr Tyr Tyr Ser Ile Lys Asp Ile Ser Val Gly Gly Met Cys Ile Cys Tyr
                265                 270                 275                 280
Gly His Ala Ser Ser Cys Pro Trp Asp Glu Glu Ala Lys Gln Leu Gln Cys Gln Cys Glu
                285                 290                 295                 300
His Asn Thr Cys Gly Glu Ser Cys Asp Arg Cys Cys Pro Gly Tyr His Gln Gln Pro Trp
                305                 310                 315                 320
Arg Pro Gly Thr Ile Ser Ser Gly Asn Glu Cys Glu Glu Cys Asn Cys His Asn Lys Ala
                325                 330                 335                 340
Lys Asp Cys Tyr Tyr Asp Ser Ser Val Ala Lys Glu Arg Arg Ser Leu Asn Thr Ala Gly
                345                 350                 355                 360
Gln Tyr Ser Gly Gly Gly Val Cys Val Asn Cys Ser Gln Asn Thr Thr Gly Ile Asn Cys
                365                 370                 375                 380
Glu Thr Cys Ile Asp Gln Tyr Tyr Arg Pro His Lys Val Ser Pro Tyr Asp Asp His Pro
                385                 390                 395                 400
Cys Arg Pro Cys Asn Cys Asp Pro Val Gly Ser Leu Ser Ser Val Cys Ile Lys Asp Asp
```

405 410 415 420
Arg His Ala Asp Leu Ala Asn Gly Lys Trp Pro Gly Gln Cys Pro Cys Arg Lys Gly Tyr
425 430 435 440
Ala Gly Asp Lys Cys Asp Arg Cys Gln Phe Gly Tyr Arg Gly Phe Pro Asn Cys Il Pro
445 450 455 460
Cys Asp Cys Arg Thr Val Gly Ser Leu Asn Glu Asp Pro Cys Ile Glu Pro Cys Leu Cys
465 470 475 480
Lys Lys Asn Val Glu Gly Lys Asn Cys Asp Arg Cys Lys Pro Gly Phe Tyr Asn Leu Lys
485 490 495 500
Glu Arg Asn Pro Glu Gly Cys Ser Glu Cys Phe Cys Phe Gly Val Ser Gly Val Cys Asp
505 510 515 520
Ser Leu Thr Trp Ser Ile Ser Gln Val Thr Asn Met Ser Gly Trp Leu Val Thr Asp Leu
525 530 535 540
Met Ser Thr Asn Lys Ile Arg Ser Gln Gln Asp Val Leu Gly Gly His Arg Gln Ile Ser
545 550 555 560
Ile Asn Asn Thr Ala Val Met Gln Arg Leu Thr Ser Thr Tyr Tyr Trp Ala Ala Pro Glu
565 570 575 580
Ala Tyr Leu Gly Asn Lys Leu Thr Ala Phe Gly Gly Phe Leu Lys Tyr Thr Val Ser Tyr
585 590 595 600
Asp Ile Pro Val Glu Thr Val Asp Ser Asp Leu Met Ser His Ala Asp Ile Ile Ile Lys
605 610 615 620
Gly Asn Gly Leu Thr Ile Ser Thr Arg Ala Glu Gly Leu Ser Leu Gln Pro Tyr Glu Glu
625 630 635 640
Tyr Phe Asn Val Val Arg Leu Val Pro Glu Asn Phe Arg Asp Phe Asn Thr Arg Arg Glu
645 650 655 660
Ile Asp Arg Asp Gln Leu Met Thr Val Leu Ala Asn Val Thr His Leu Leu Ile Arg Ala
665 670 675 680
Asn Tyr Asn Ser Ala Lys Met Ala Leu Tyr Arg Leu Asp Ser Val Ser Leu Asp Ile Ala
685 690 695 700
Ser Pro Asn Ala Ile Asp Leu Ala Val Ala Ala Asp Val Glu His Cys Glu Cys Pro Gln
705 710 715 720
Gly Tyr Thr Gly Thr Ser Cys Glu Ala Cys Leu Pro Gly Tyr Tyr Arg Val Asp Gly Ile
725 730 735 740
Leu Phe Gly Gly Ile Cys Gln Pro Cys Glu Cys His Gly His Ala Ser Glu Cys Asp Ile
745 750 755 760
His Gly Ile Cys Ser Val Cys Thr His Asn Thr Thr Gly Asp His Cys Glu Gln Cys Leu
765 770 775 780
Pro Gly Phe Tyr Gly Thr Pro Ser Arg Gly Thr Pro Gly Asp Cys Gln Pro Cys Ala Cys
785 790 795 800
Pro Leu Ser Ile Asp Ser Asn Asn Phe Ser Pro Thr Cys His Leu Thr Asp Gly Glu Glu
805 810 815 820
Val Val Cys Asp Gln Cys Ala Pro Gly Tyr Ser Gly Ser Trp Cys Glu Arg Cys Ala Asp
825 830 835 840
Gly Tyr Tyr Gly Asn Pro Thr Val Pro Gly Gly Thr Cys Val Pro Cys Asn Cys Ser Gly
845 850 855 860
Asn Val Asp Pro Leu Glu Ala Gly His Cys Asp Ser Val Thr Gly Glu Cys Leu Lys Cys
865 870 875 880
Leu Trp Asn Thr Asp Gly Ala His Cys Glu Arg Cys Ala Asp Gly Phe Tyr Gly Asp Ala
885 890 895 900
Val Thr Ala Lys Asn Cys Arg Ala Cys Asp Cys His Glu Asn Gly Ser Leu Ser Gly Val
905 910 915 920
Cys His Leu Glu Thr Gly Leu Cys Asp Cys Lys Pro His Val Thr Gly Gln Gln Cys Asp
925 930 935 940
Gln Cys Leu Ser Gly Tyr Tyr Gly Leu Asp Thr Gly Leu Gly Cys Val Pro Cys Asn Cys
945 950 955 960
Ser Val Glu Gly Ser Val Ser Asp Asn Cys Thr Glu Glu Gly Gln Cys His Cys Gly Pro
965 970 975 980
Gly Val Ser Gly Lys Gln Cys Asp Arg Cys Ser His Gly Phe Tyr Ala Phe Gln Asp Gly
985 990 995 1000
Gly Cys Thr Pro Cys Asp Cys Ala His Thr Gln Asn Asn Cys Asp Pro Ala Ser Gly Glu
1005 1010 1015 1020
Cys Leu Cys Pro Pro His Thr Gln Gly Leu Lys Cys Glu Glu Cys Glu Glu Ala Tyr Trp
1025 1030 1035 1040

Gly Leu Asp Pro Glu Gln Gly Cys Gln Ala Cys Asn Cys Ser Ala Val Gly Ser Thr Ser
1045 1050 1055 1060
Ala Gln Cys Asp Val Leu Ser Gly His Cys Pro Cys Lys Lys Gly Phe Gly Gly Gln Ser
1065 1070 1075 1080
Cys His Gln Cys Ser Leu Gly Tyr Arg Ser Phe Pro Asp Cys Val Pro Cys Gly Cys Asp
1085 1090 1095 1100
Leu Arg Gly Thr Leu Pro Asp Thr Cys Asp Leu Glu Gln Gly Leu Cys Ser Cys Ser Glu
1105 1110 1115 1120
Asp Ser Gly Thr Cys Ser Cys Lys Glu Asn Val Val Gly Pro Gln Cys Ser Lys Cys Gln
1125 1130 1135 1140
Ala Gly Thr Phe Ala Leu Arg Gly Asp Asn Pro Gln Gly Cys Ser Pro Cys Phe Cys Phe
1145 1150 1155 1160
Gly Leu Ser Gln Leu Cys Ser Glu Leu Glu Gly Tyr Val Arg Thr Leu Ile Thr Leu Ala
1165 1170 1175 1180
Ser Asp Gln Pro Leu Leu His Val Val Ser Gln Ser Asn Leu Lys Gly Thr Ile Glu Gly
1185 1190 1195 1200
Val His Phe Gln Pro Pro Asp Thr Leu Leu Asp Ala Glu Ala Val Arg Gln His Ile Tyr
1205 1210 1215 1220
Ala Glu Pro Phe Tyr Trp Arg Leu Pro Lys Gln Phe Gln Gly Asp Gln Leu Leu Ala Tyr
1225 1230 1235 1240
Gly Gly Lys Leu Gln Tyr Ser Val Ala Phe Tyr Ser Thr Leu Gly Thr Gly Thr Ser Asn
1245 1250 1255 1260
Tyr Glu Pro Gln Val Leu Ile Lys Gly Gly Arg Ala Arg Lys His Val Ile Tyr Met Asp
1265 1270 1275 1280
Ala Pro Ala Pro Glu Asn Gly Val Arg Gln Asp Tyr Glu Val Gln Met Lys Glu Glu Phe
1285 1290 1295 1300
Trp Lys Tyr Phe Asn Ser Val Ser Glu Lys His Val Thr His Ser Asp Phe Met Ser Val
1305 1310 1315 1320
Leu Ser Asn Ile Asp Tyr Ile Leu Ile Lys Ala Ser Tyr Gly Gln Gly Leu Gln Gln Ser
1325 1330 1335 1340
Arg Ile Ala Asn Ile Ser Met Glu Val Gly Arg Lys Ala Val Glu Leu Pro Ala Glu Gly
1345 1350 1355 1360
Glu Ala Ala Leu Leu Leu Glu Leu Cys Val Cys Pro Pro Gly Thr Ala Gly His Ser Cys
1365 1370 1375 1380
Gln Asp Cys Ala Pro Gly Tyr Tyr Arg Glu Lys Leu Pro Glu Ser Gly Gly Arg Gly Pro
1385 1390 1395 1400
Arg Pro Leu Leu Ala Pro Cys Val Pro Cys Asn Cys Asn Asn His Ser Asp Val Cys Asp
1405 1410 1415 1420
Pro Glu Thr Gly Lys Cys Leu Ser Cys Arg Asp His Thr Ser Gly Asp His Cys Glu Leu
1425 1430 1435 1440
Cys Ala Ser Gly Tyr Tyr Gly Lys Val Thr Gly Leu Pro Gly Asp Cys Thr Pro Cys Thr
1445 1450 1455 1460
Cys Pro His His Pro Pro Phe Ser Phe Ser Pro Thr Cys Val Val Glu Gly Asp Ser Asp
1465 1470 1475 1480
Phe Arg Cys Asn Ala Cys Leu Pro Gly Tyr Glu Gly Gln Tyr Cys Glu Arg Cys Ser Ala
1485 1490 1495 1500
Gly Tyr His Gly Asn Pro Arg Ala Ala Gly Gly Ser Cys Gln Thr Cys Asp Cys Asn Pro
1505 1510 1515 1520
Gln Gly Ser Val His Ser Asp Cys Asp Arg Ala Ser Gly Gln Cys Val Cys Lys Pro Gly
1525 1530 1535 1540
Ala Thr Gly Leu His Cys Glu Lys Cys Leu Pro Arg His Ile Leu Met Glu Ser Asp Cys
1545 1550 1555 1560
Val Ser Cys Asp Asp Asp Cys Val Gly Pro Leu Leu Asn Asp Leu Asp Ser Val Gly Asp
1565 1570 1575 1580
Ala Val Leu Ser Leu Asn Leu Thr Gly Val Ser Pro Ala Pro Tyr Gly Ile Leu Glu Asn
1585 1590 1595 1600
Leu Glu Asn Thr Thr Lys Tyr Phe Gln Arg Tyr Leu Ile Lys Glu Asn Ala Lys Lys Ile
1605 1610 1615 1620
Arg Ala Glu Ile Gln Leu Glu Gly Ile Ala Glu Gln Thr Glu Asn Leu Gln Lys Glu Leu
1625 1630 1635 1640
Thr Arg Val Leu Ala Arg His Gln Lys Val Asn Ala Glu Met Glu Arg Thr Ser Asn Gly
1645 1650 1655 1660
Thr Gln Ala Leu Ala Thr Phe Ile Glu Gln Leu His Ala Asn Ile Lys Glu Ile Thr Glu

1665 1670 1675 1680
Lys Val Ala Thr Leu Asn Gln Thr Ala Arg Lys Asp Phe Gln Pro Pro Val Ser Ala Leu
1685 1690 1695 1700
Gln Ser Met His Gln Asn Ile Ser Ser Leu Leu Gly Leu Ile Lys Glu Arg Asn Phe Thr
1705 1710 1715 1720
Glu Met Gln Gln Asn Ala Thr Leu Glu Leu Lys Ala Ala Lys Asp Leu Leu Ser Arg Ile
1725 1730 1735 1740
Gln Lys Arg Phe Gln Lys Pro Gln Glu Lys Leu Lys Ala Leu Lys Glu Ala Asn Ser Leu
1745 1750 1755 1760
Leu Ser Asn His Ser Glu Lys Leu Gln Ala Ala Glu Glu Leu Leu Lys Glu Ala Gly Ser
1765 1770 1775 1780
Lys Thr Gln Glu Ser Asn Leu Leu Leu Leu Leu Val Lys Ala Asn Leu Lys Glu Glu Phe
1785 1790 1795 1800
Gln Glu Lys Lys Leu Arg Val Gln Glu Glu Gln Asn Val Thr Ser Glu Leu Ile Ala Lys
1805 1810 1815 1820
Gly Arg Glu Trp Val Asp Ala Ala Gly Thr His Thr Ala Ala Ala Gln Asp Thr Leu Thr
1825 1830 1835 1840
Gln Leu Glu His His Arg Asp Glu Leu Leu Leu Trp Ala Arg Lys Ile Arg Ser His Val
1845 1850 1855 1860
Asp Asp Leu Val Met Gln Met Ser Lys Arg Arg Ala Arg Asp Leu Val His Arg Ala Glu
1865 1870 1875 1880
Gln His Ala Ser Glu Leu Gln Ser Arg Ala Gly Ala Leu Asp Arg Asp Leu Glu Asn Val
1885 1890 1895 1900
Arg Asn Val Ser Leu Asn Ala Thr Ser Ala Ala His Val His Ser Asn Ile Gln Thr Leu
1905 1910 1915 1920
Thr Glu Glu Ala Glu Met Leu Ala Ala Asp Ala His Lys Thr Ala Asn Lys Thr Asp Leu
1925 1930 1935 1940
Ile Ser Glu Ser Leu Ala Ser Arg Gly Lys Ala Val Leu Gln Arg Ser Ser Arg Phe Leu
1945 1950 1955 1960
Lys Glu Ser Val Gly Thr Arg Arg Lys Gln Gln Gly Ile Thr Met Lys Leu Asp Glu Leu
1965 1970 1975 1980
Lys Asn Leu Thr Ser Gln Phe Gln Glu Ser Val Asp Asn Ile Thr Lys Gln Ala Asn Asp
1985 1990 1995 2000
Ser Leu Ala Met Leu Arg Glu Ser Pro Gly Gly Met Arg Glu Lys Gly Arg Lys Ala Arg
2005 2010 2015 2020
Glu Leu Ala Ala Ala Ala Asn Glu Ser Ala Val Lys Thr Leu Glu Asp Val Leu Ala Leu
2025 2030 2035 2040
Ser Leu Arg Val Phe Asn Thr Ser Glu Asp Leu Ser Arg Val Asn Ala Thr Val Gln Glu
2045 2050 2055 2060
Thr Asn Asp Leu Leu His Asn Ser Thr Met Thr Thr Leu Leu Ala Gly Arg Lys Met Lys
2065 2070 2075 2080
Asp Met Glu Met Gln Ala Asn Leu Leu Leu Asp Arg Leu Lys Pro Leu Lys Thr Leu Glu
2085 2090 2095 2100
Glu Asn Leu Ser Arg Asn Leu Ser Glu Ile Lys Leu Leu Ile Ser Arg Ala Arg Lys Gln
2105 2110 2115 2120
Ala Ala Ser Ile Lys Val Ala Val Ser Ala Asp Arg Asp Cys Ile Arg Ala Tyr Gln Pro
2125 2130 2135 2140
Gln Thr Ser Ser Thr Asn Tyr Asn Thr Leu Ile Leu Asn Val Lys Thr Gln Glu Pro Asp
2145 2150 2155 2160
Asn Leu Leu Phe Tyr Leu Gly Ser Ser Ser Ser Ser Asp Phe Leu Ala Val Glu Met Arg
2165 2170 2175 2180
Arg Gly Lys Val Ala Phe Leu Trp Asp Leu Gly Ser Gly Ser Thr Arg Leu Glu Phe Pro
2185 2190 2195 2200
Glu Val Ser Ile Asn Asn Asn Arg Trp His Ser Ile Tyr Ile Thr Arg Phe Gly Asn Met
2205 2210 2215 2220
Gly Ser Leu Ser Val Lys Glu Ala Ser Ala Ala Glu Asn Pro Pro Val Arg Thr Ser Lys
2225 2230 2235 2240
Ser Pro Gly Pro Ser Lys Val Leu Asp Ile Asn Asn Ser Thr Leu Met Phe Val Gly Gly
2245 2250 2255 2260
Leu Gly Gly Gln Ile Lys Lys Ser Pro Ala Val Lys Val Thr His Phe Lys Gly Cys Met
2265 2270 2275 2280
Gly Glu Ala Phe Leu Asn Gly Lys Ser Ile Gly Leu Trp Asn Tyr Ile Glu Arg Glu Gly
2285 2290 2295 2300

Lys Cys Asn Gly Cys Phe Gly Ser Ser Gln Asn Glu Asp Ser Ser Phe His Phe Asp Gly
2305 2310 2315 2320
Ser Gly Tyr Ala Met Val Glu Lys Thr Leu Arg Pro Thr Val Thr Gln Ile Val Ile Leu
2325 2330 2335 2340
Phe Ser Thr Phe Ser Pro Asn Gly Leu Leu Phe Tyr Leu Ala Ser Asn Gly Thr Lys Asp
2345 2350 2355 2360
Phe Leu Ser Ile Glu Leu Val Arg Gly Arg Val Lys Val Met Val Asp Leu Gly Ser Gly
2365 2370 2375 2380
Pro Leu Thr Leu Met Thr Asp Arg Arg Tyr Asn Asn Gly Thr Trp Tyr Lys Ile Ala Phe
2385 2390 2395 2400
Gln Arg Asn Arg Lys Gln Gly Leu Leu Ala Val Phe Asp Ala Tyr Asp Thr Ser Asp Lys
2405 2410 2415 2420
Glu Thr Lys Gln Gly Glu Thr Pro Gly Ala Ala Ser Asp Leu Asn Arg Leu Glu Lys Asp
2425 2430 2435 2440
Leu Ile Tyr Val Gly Gly Leu Pro His Ser Lys Ala Val Arg Lys Gly Val Ser Ser Arg
2445 2450 2455 2460
Ser Tyr Val Gly Cys Ile Lys Asn Leu Glu Ile Ser Arg Ser Thr Phe Asp Leu Leu Arg
2465 2470 2475 2480
Asn Ser Tyr Gly Val Arg Lys Gly Cys Ala Leu Glu Pro Ile Gln Ser Val Ser Phe Leu
2485 2490 2495 2500
Arg Gly Gly Tyr Val Glu Met Pro Pro Lys Ser Leu Ser Pro Glu Ser Ser Leu Leu Ala
2505 2510 2515 2520
Thr Phe Ala Thr Lys Asn Ser Ser Gly Ile Leu Leu Val Ala Leu Gly Lys Asp Ala Glu
2525 2530 2535 2540
Glu Ala Gly Gly Ala Gln Ala His Val Pro Phe Phe Ser Ile Met Leu Leu Glu Gly Arg
2545 2550 2555 2560
Ile Glu Val His Val Asn Ser Gly Asp Gly Thr Ser Leu Arg Lys Ala Leu Leu His Ala
2565 2570 2575 2580
Pro Thr Gly Ser Tyr Ser Asp Gly Gln Glu His Ser Ile Ser Leu Val Arg Asn Arg Arg
2585 2590 2595 2600
Val Ile Thr Ile Gln Val Asp Glu Asn Ser Pro Val Glu Met Lys Leu Gly Pro Leu Thr
2605 2610 2615 2620
Glu Gly Lys Thr Ile Asp Ile Ser Asn Leu Tyr Ile Gly Gly Leu Pro Glu Asp Lys Ala
2625 2630 2635 2640
Thr Pro Met Leu Lys Met Arg Thr Ser Phe His Gly Cys Ile Lys Asn Val Val Leu Asp
2645 2650 2655 2660
Ala Gln Leu Leu Asp Phe Thr His Ala Thr Gly Ser Glu Gln Val Glu Leu Asp Thr Cys
2665 2670 2675 2680
Leu Leu Ala Glu Glu Pro Met Gln Ser Leu His Arg Glu His Gly Glu Leu Pro Pro Glu
2685 2690 2695 2700
Pro Pro Thr Leu Pro Gln Pro Glu Leu Cys Ala Val Asp Thr Ala Pro Gly Tyr Val Ala
2705 2710 2715 2720
Gly Ala His Gln Phe Gly Leu Ser Gln Asn Ser His Leu Val Leu Pro Leu Asn Gln Ser
2725 2730 2735 2740
Asp Val Arg Lys Arg Leu Gln Val Gln Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu
2745 2750 2755 2760
Ile Tyr Tyr Val Ala His Gln Asn Gln Met Asp Tyr Ala Thr Leu Gln Leu Gln Glu Gly
2765 2770 2775 2780
Arg Leu His Phe Met Phe Asp Leu Gly Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu
2785 2790 2795 2800
Leu Ser Asp Gly Lys Trp His Thr Val Lys Thr Glu Tyr Ile Lys Arg Lys Ala Phe Met
2805 2810 2815 2820
Thr Val Asp Gly Gln Glu Ser Pro Ser Val Thr Val Val Gly Asn Ala Thr Thr Leu Asp
2825 2830 2835 2840
Val Glu Arg Lys Leu Tyr Leu Gly Gly Leu Pro Ser His Tyr Arg Ala Arg Asn Ile Gly
2845 2850 2855 2860
Thr Ile Thr His Ser Ile Pro Ala Cys Ile Gly Glu Ile Met Val Asn Gly Gln Gln Leu
2865 2870 2875 2880
Asp Lys Asp Arg Pro Leu Ser Ala Ser Ala Val Asp Arg Cys Tyr Val Val Ala Gln Glu
2885 2890 2895 2900
Gly Thr Phe Phe Glu Gly Ser Gly Tyr Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Arg
2905 2910 2915 2920
Leu Asp Leu Asn Ile Thr Leu Glu Phe Arg Thr Thr Ser Lys Asn Gly Val Leu Leu Gly

```
                2925                2930                2935                2940
Ile Ser Ser Ala Lys Val Asp Ala Ile Gly Leu Glu Ile Val Asp Gly Lys Val Leu Phe
                2945                2950                2955                2960
His Val Asn Asn Gly Ala Gly Arg Ile Thr Ala Thr Tyr Gln Pro Arg Ala Ala Arg Ala
                2965                2970                2975                2980
Leu Cys Asp Gly Lys Trp His Thr Leu Gln Ala His Lys Ser Lys His Arg Ile Val Leu
                2985                2990                2995                3000
Thr Val Asp Gly Asn Ser Val Arg Ala Glu Ser Pro His Thr His Ser Thr Ser Ala Asp
                3005                3010                3015                3020
Thr Asn Asp Pro Ile Tyr Val Gly Gly Tyr Pro Ala His Ile Lys Gln Asn Cys Leu Ser
                3025                3030                3035                3040
Ser Arg Ala Ser Phe Arg Gly Cys Val Arg Asn Leu Arg Leu Ser Arg Gly Ser Gln Val
                3045                3050                3055                3060
Gln Ser Leu Asp Leu Ser Arg Ala Phe Asp Leu Gln Gly Val Phe Pro His Ser Cys Pro
                3065                3070                3075                3080
Gly Pro Glu Pro
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3075 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P25391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Arg Gly Gly Val Leu Leu Val Leu Leu Leu Cys Val Ala Ala Gln Cys Arg Gln Arg
1               5                   10                  15                  20
Gly Leu Phe Pro Ala Ile Leu Asn Leu Ala Ser Asn Ala His Ile Ser Thr Asn Ala Thr
                25                  30                  35                  40
Cys Gly Glu Lys Gly Pro Glu Met Phe Cys Lys Leu Val Glu His Val Pro Gly Arg Pro
                45                  50                  55                  60
Val Arg Asn Pro Gln Cys Arg Ile Cys Asp Gly Asn Ser Ala Asn Pro Arg Glu Arg His
                65                  70                  75                  80
Pro Ile Ser His Ala Ile Asp Gly Thr Asn Asn Trp Trp Gln Ser Pro Ser Ile Gln Asn
                85                  90                  95                  100
Gly Arg Glu Tyr His Trp Val Thr Ile Thr Leu Asp Leu Arg Gln Val Phe Gln Val Ala
                105                 110                 115                 120
Tyr Val Ile Ile Lys Ala Ala Asn Ala Pro Arg Pro Gly Asn Trp Ile Leu Glu Arg Ser
                125                 130                 135                 140
Leu Asp Gly Thr Thr Phe Ser Pro Trp Gln Tyr Tyr Ala Val Ser Asp Ser Glu Cys Leu
                145                 150                 155                 160
S r Arg Tyr Asn Ile Thr Pro Arg Arg Gly Pro Pro Thr Tyr Arg Ala Asp Asp Glu Val
                165                 170                 175                 180
Ile Cys Thr Ser Tyr Tyr Ser Arg Leu Val Pro Leu Glu His Gly Glu Ile His Thr Ser
                185                 190                 195                 200
Leu Ile Asn Gly Arg Pro Ser Ala Asp Asp Leu Ser Pro Lys Leu Leu Glu Phe Thr Ser
                205                 210                 215                 220
Ala Arg Tyr Ile Arg Leu Arg Leu Gln Arg Ile Arg Thr Leu Asn Ala Asp Leu Met Thr
                225                 230                 235                 240
Leu Ser His Arg Glu Pro Lys Glu Leu Asp Pro Ile Val Thr Arg Arg Tyr Tyr Tyr Ser
                245                 250                 255                 260
Ile Lys Asp Ile Ser Val Gly Gly Met Cys Ile Cys Tyr Gly His Ala Ser Ser Cys Pro
                265                 270                 275                 280
Trp Asp Glu Thr Thr Lys Lys Leu Gln Cys Gln Cys Glu His Asn Thr Cys Gly Glu Ser
```

```
                285                 290                 295                 300
Cys Asn Arg Cys Cys Pro Gly Tyr His Gln Gln Pro Trp Arg Pro Gly Thr Val Ser Ser
            305                 310                 315                 320
Gly Asn Thr Cys Glu Ala Cys Asn Cys His Asn Lys Ala Lys Asp Cys Tyr Tyr Asp Glu
                325                 330                 335                 340
Ser Val Ala Lys Gln Lys Lys Ser Leu Asn Thr Ala Gly Gln Phe Arg Gly Gly Gly Val
                345                 350                 355                 360
Cys Ile Asn Cys Leu Gln Asn Thr Met Gly Ile Asn Cys Glu Thr Cys Ile Asp Gly Tyr
                365                 370                 375                 380
Tyr Arg Pro His Lys Val Ser Pro Tyr Glu Asp Glu Pro Cys Arg Pro Cys Asn Cys Asp
                385                 390                 395                 400
Pro Val Gly Ser Leu Ser Ser Val Cys Ile Lys Asp Asp Leu His Ser Asp Leu His Asn
                405                 410                 415                 420
Gly Lys Gln Pro Gly Gln Cys Pro Cys Lys Glu Gly Tyr Thr Gly Glu Lys Cys Asp Arg
                425                 430                 435                 440
Cys Gln Leu Gly Tyr Lys Asp Tyr Pro Thr Cys Val Ser Cys Gly Cys Asn Pro Val Gly
                445                 450                 455                 460
Ser Ala Ser Asp Glu Pro Cys Thr Gly Pro Cys Val Cys Lys Glu Asn Val Glu Gly Lys
                465                 470                 475                 480
Ala Cys Asp Arg Cys Lys Pro Gly Phe Tyr Asn Leu Lys Glu Lys Asn Pro Arg Gly Cys
                485                 490                 495                 500
Ser Glu Cys Phe Cys Phe Gly Val Ser Asp Val Cys Ser Ser Leu Ser Trp Pro Val Gly
                505                 510                 515                 520
Gln Val Asn Ser Met Ser Gly Trp Leu Val Thr Asp Leu Ile Ser Pro Arg Lys Ile Pro
                525                 530                 535                 540
Ser Gln Gln Asp Ala Leu Gly Gly Arg His Gln Val Ser Ile Asn Asn Thr Ala Val Met
                545                 550                 555                 560
Gln Arg Leu Ala Pro Lys Tyr Tyr Trp Ala Ala Pro Glu Ala Tyr Leu Gly Asn Lys Leu
                565                 570                 575                 580
Thr Ala Phe Gly Gly Phe Leu Lys Tyr Thr Val Ser Tyr Asp Ile Pro Val Glu Thr Val
                585                 590                 595                 600
Asp Ser Asn Leu Met Ser His Ala Asp Val Ile Ile Lys Gly Asn Gly Leu Thr Leu Ser
                605                 610                 615                 620
Thr Gln Ala Glu Gly Leu Ser Leu Gln Pro Tyr Glu Glu Tyr Leu Asn Val Val Arg Leu
                625                 630                 635                 640
Val Pro Glu Asn Phe Gln Asp Phe His Ser Lys Arg Gln Ile Asp Arg Asp Gln Leu Met
                645                 650                 655                 660
Thr Val Leu Ala Asn Val Thr His Leu Leu Ile Arg Ala Thr Tyr Asn Ser Ala Lys Met
                665                 670                 675                 680
Ala Leu Tyr Arg Leu Glu Ser Val Ser Leu Asp Ile Ala Ser Ser Asn Ala Ile Asp Leu
                685                 690                 695                 700
Val Val Ala Ala Asp Val Glu His Cys Glu Cys Pro Gln Gly Tyr Thr Gly Thr Ser Cys
                705                 710                 715                 720
Glu Ser Cys Leu Ser Gly Tyr Tyr Arg Val Asp Gly Ile Leu Phe Gly Gly Ile Cys Gln
                725                 730                 735                 740
Pro Cys Glu Cys His Gly His Ala Ala Glu Cys Asn Val His Gly Val Cys Ile Ala Cys
                745                 750                 755                 760
Ala His Asn Thr Thr Gly Val His Cys Glu Gln Cys Leu Pro Gly Phe Tyr Gly Glu Pro
                765                 770                 775                 780
Ser Arg Gly Thr Pro Gly Asp Cys Gln Pro Cys Ala Cys Pro Leu Thr Ile Ala Ser Asn
                785                 790                 795                 800
Asn Phe Ser Pro Thr Cys His Leu Asn Asp Gly Asp Glu Val Val Cys Asp Trp Cys Ala
                805                 810                 815                 820
Pro Gly Tyr Ser Gly Ala Trp Cys Glu Arg Cys Ala Asp Gly Tyr Tyr Gly Asn Pro Thr
                825                 830                 835                 840
Val Pro Gly Glu Ser Cys Val Pro Cys Asp Cys Ser Gly Asn Val Asp Pro Ser Glu Ala
                845                 850                 855                 860
Gly His Cys Asp Ser Val Thr Gly Glu Cys Leu Lys Cys Leu Gly Asn Thr Asp Gly Ala
                865                 870                 875                 880
His Cys Glu Arg Cys Ala Asp Gly Phe Tyr Gly Asp Ala Val Thr Ala Lys Asn Cys Arg
                885                 890                 895                 900
Ala Cys Glu Cys His Val Lys Gly Ser His Ser Ala Val Cys His Leu Glu Thr Gly Leu
                905                 910                 915                 920
```

Cys Asp Cys Lys Pro Asn Val Thr Gly Gln Gln Cys Asp Gln Cys Leu His Gly Tyr Tyr
925 930 935 940
Gly Leu Asp Ser Gly His Gly Cys Arg Pro Cys Asn Cys Ser Val Ala Gly Ser Val Ser
945 950 955 960
Asp Gly Cys Thr Asp Glu Gly Gln Cys His Cys Val Pro Gly Val Ala Gly Lys Arg Cys
965 970 975 980
Asp Arg Cys Ala His Gly Phe Tyr Ala Tyr Gln Asp Gly Ser Cys Thr Pro Cys Asp Cys
985 990 995 1000
Pro His Thr Gln Asn Thr Cys Asp Pro Glu Thr Gly Glu Cys Val Cys Pro Pro His Thr
1005 1010 1015 1020
Gln Gly Gly Lys Cys Glu Glu Cys Glu Asp Gly His Trp Gly Tyr Asp Ala Glu Val Gly
1025 1030 1035 1040
Cys Gln Ala Cys Asn Cys Ser Leu Val Gly Ser Thr His His Arg Cys Asp Val Val Thr
1045 1050 1055 1060
Gly His Cys Gln Cys Lys Ser Lys Phe Gly Gly Arg Ala Cys Asp Gln Cys Ser Leu Gly
1065 1070 1075 1080
Tyr Arg Asp Phe Pro Asp Cys Val Pro Cys Asp Cys Asp Leu Arg Gly Thr Ser Gly Asp
1085 1090 1095 1100
Ala Cys Asn Leu Glu Gln Gly Leu Cys Gly Cys Val Glu Glu Thr Gly Ala Cys Pro Cys
1105 1110 1115 1120
Lys Glu Asn Val Phe Gly Pro Gln Cys Asn Glu Cys Arg Glu Gly Thr Phe Ala Leu Arg
1125 1130 1135 1140
Ala Asp Asn Pro Leu Gly Cys Ser Pro Cys Phe Cys Ser Gly Leu Ser His Leu Cys Ser
1145 1150 1155 1160
Glu Leu Glu Asp Tyr Val Arg Thr Pro Val Thr Leu Gly Ser Asp Gln Pro Leu Leu Arg
1165 1170 1175 1180
Val Val Ser Gln Ser Asn Leu Arg Gly Thr Thr Glu Gly Val Tyr Tyr Gln Ala Pro Asp
1185 1190 1195 1200
Phe Leu Leu Asp Ala Ala Thr Val Arg Gln His Ile Arg Ala Glu Pro Phe Tyr Trp Arg
1205 1210 1215 1220
Leu Pro Gln Gln Phe Gln Gly Asp Gln Leu Met Ala Tyr Gly Gly Lys Leu Lys Tyr Ser
1225 1230 1235 1240
Val Ala Phe Tyr Ser Leu Asp Gly Val Gly Thr Ser Asn Phe Glu Pro Gln Val Leu Ile
1245 1250 1255 1260
Lys Gly Gly Arg Ile Arg Lys Gln Val Ile Tyr Met Asp Ala Pro Ala Pro Glu Asn Gly
1265 1270 1275 1280
Val Arg Gln Glu Gln Glu Val Ala Met Arg Glu Asn Phe Trp Lys Tyr Phe Asn Ser Val
1285 1290 1295 1300
Ser Glu Lys Pro Val Thr Arg Glu Asp Phe Met Ser Val Leu Ser Asp Ile Glu Tyr Ile
1305 1310 1315 1320
L u Ile Lys Ala Ser Tyr Gly Gln Gly Leu Gln Gln Ser Arg Ile Ser Asp Ile Ser Val
1325 1330 1335 1340
Glu Val Gly Arg Lys Ala Glu Lys Leu His Pro Glu Glu Glu Val Ala Ser Leu Leu Glu
1345 1350 1355 1360
Asn Cys Val Cys Pro Pro Gly Thr Val Gly Phe Ser Cys Gln Asp Cys Ala Pro Gly Tyr
1365 1370 1375 1380
His Arg Gly Lys Leu Pro Ala Gly Ser Asp Arg Gly Pro Arg Pro Leu Val Ala Pro Cys
1385 1390 1395 1400
Val Pro Cys Ser Cys Asn Asn His Ser Asp Thr Cys Asp Pro Asn Thr Gly Lys Cys Leu
1405 1410 1415 1420
Asn Cys Gly Asp Asn Thr Ala Gly Asp His Cys Asp Val Cys Thr Ser Gly Tyr Tyr Gly
1425 1430 1435 1440
Lys Val Thr Gly Ser Ala Ser Asp Cys Ala Leu Cys Ala Cys Pro His Ser Pro Pro Ala
1445 1450 1455 1460
Ser Phe Ser Pro Thr Cys Val Leu Glu Gly Asp His Asp Phe Arg Cys Asp Ala Cys Leu
1465 1470 1475 1480
Leu Gly Tyr Glu Gly Lys His Cys Glu Arg Cys Ser Ser Ser Tyr Tyr Gly Asn Pro Gln
1485 1490 1495 1500
Thr Pro Gly Gly Ser Cys Gln Lys Cys Asp Cys Asn Arg His Gly Ser Val His Gly Asp
1505 1510 1515 1520
Cys Asp Arg Thr Ser Gly Gln Cys Val Cys Arg Leu Gly Ala Ser Gly Leu Arg Cys Asp
1525 1530 1535 1540
Glu Cys Glu Pro Arg His Ile Leu Met Glu Thr Asp Cys Val Ser Cys Asp Asp Glu Cys

1545 1550 1555 1560
Val Gly Val Leu Leu Asn Asp Leu Asp Glu Ile Gly Asp Ala Val Leu Ser Leu Asn Leu
1565 1570 1575 1580
Thr Gly Ile Ile Pro Val Pro Tyr Gly Ile Leu Ser Asn Leu Glu Asn Thr Thr Lys Tyr
1585 1590 1595 1600
Leu Gln Glu Ser Leu Leu Lys Glu Asn Met Gln Lys Asp Leu Gly Lys Ile Lys Leu Glu
1605 1610 1615 1620
Gly Val Ala Glu Glu Thr Asp Asn Leu Gln Lys Lys Leu Thr Arg Met Leu Ala Ser Thr
1625 1630 1635 1640
Gln Lys Val Asn Arg Ala Thr Glu Arg Ile Phe Lys Glu Ser Gln Asp Leu Ala Val Ala
1645 1650 1655 1660
Ile Glu Arg Leu Gln Met Ser Ile Thr Glu Ile Met Glu Lys Thr Thr Leu Asn Gln Thr
1665 1670 1675 1680
Leu Asp Glu Asp Phe Leu Leu Pro Asn Ser Thr Leu Gln Asn Met Gln Gln Asn Gly Thr
1685 1690 1695 1700
Ser Leu Leu Glu Ile Met Gln Ile Arg Asp Phe Thr Gln Leu His Gln Asn Ala Thr Leu
1705 1710 1715 1720
Glu Leu Lys Ala Ala Glu Asp Leu Leu Ser Gln Ile Gln Glu Asn Tyr Gln Lys Pro Leu
1725 1730 1735 1740
Glu Glu Leu Glu Val Leu Lys Glu Ala Ala Ser His Val Leu Ser Lys His Asn Asn Glu
1745 1750 1755 1760
Leu Lys Ala Ala Glu Ala Leu Val Arg Glu Ala Glu Ala Lys Met Gln Glu Ser Asn His
1765 1770 1775 1780
Leu Leu Leu Met Val Asn Ala Asn Leu Arg Glu Phe Ser Asp Lys Lys Leu His Val Gln
1785 1790 1795 1800
Glu Glu Gln Asn Leu Thr Ser Glu Leu Ile Val Gln Gly Arg Gly Leu Ile Asp Ala Ala
1805 1810 1815 1820
Ala Ala Gln Thr Asp Ala Val Gln Asp Ala Leu Glu His Leu Glu Asp His Gln Asp Lys
1825 1830 1835 1840
Leu Leu Leu Trp Ser Ala Lys Ile Arg His His Ile Asp Asp Leu Val Met His Met Ser
1845 1850 1855 1860
Gln Arg Asn Ala Val Asp Leu Val Tyr Arg Ala Glu Asp His Ala Thr Glu Phe Gln Arg
1865 1870 1875 1880
Leu Ala Asp Val Leu Tyr Ser Gly Leu Glu Asn Ile Arg Asn Val Ser Leu Asn Ala Thr
1885 1890 1895 1900
Ser Ala Ala Tyr Val His Tyr Asn Ile Gln Ser Leu Ile Glu Glu Ser Glu Glu Leu Ala
1905 1910 1915 1920
Arg Asp Ala His Arg Thr Val Thr Glu Thr Ser Leu Leu Ser Glu Ser Leu Val Ser Asn
1925 1930 1935 1940
Gly Lys Ala Ala Val Gln Arg Ser Ser Arg Phe Leu Lys Glu Gly Asn Asn Leu Ser Arg
1945 1950 1955 1960
Lys Leu Pro Gly Ile Ala Leu Glu Leu Ser Glu Leu Arg Asn Lys Thr Asn Arg Phe Gln
1965 1970 1975 1980
Glu Asn Ala Val Glu Ile Thr Arg Gln Thr Asn Glu Ser Leu Leu Ile Leu Arg Ala Ile
1985 1990 1995 2000
Pro Glu Gly Ile Arg Asp Lys Gly Ala Lys Thr Lys Glu Leu Ala Thr Ser Ala Ser Gln
2005 2010 2015 2020
Ser Ala Val Ser Thr Leu Arg Asp Val Ala Gly Leu Ser Gln Glu Leu Leu Asn Thr Ser
2025 2030 2035 2040
Ala Ser Leu Ser Arg Val Asn Thr Thr Leu Arg Glu Thr His Gln Leu Leu Gln Asp Ser
2045 2050 2055 2060
Thr Met Ala Thr Leu Leu Ala Gly Arg Lys Val Lys Asp Val Glu Ile Gln Ala Asn Leu
2065 2070 2075 2080
Leu Phe Asp Arg Leu Lys Pro Leu Lys Met Leu Glu Glu Asn Leu Ser Arg Asn Leu Ser
2085 2090 2095 2100
Glu Ile Lys Leu Leu Ile Ser Gln Ala Arg Lys Gln Ala Ala Ser Ile Lys Val Ala Val
2105 2110 2115 2120
Ser Ala Asp Arg Asp Cys Ile Arg Ala Tyr Gln Pro Gln Ile Ser Ser Thr Asn Tyr Asn
2125 2130 2135 2140
Thr Leu Thr Leu Asn Val Lys Thr Gln Glu Pro Asp Asn Leu Leu Phe Tyr Leu Gly Ser
2145 2150 2155 2160
Ser Thr Ala Ser Asp Phe Leu Ala Val Glu Met Arg Arg Gly Arg Val Ala Phe Leu Trp
2165 2170 2175 2180

Asp Leu Gly Ser Gly Ser Thr Arg Leu Glu Phe Pro Asp Phe Pro Ile Asp Asp Asn Arg
2185 2190 2195 2200
Trp His Ser Ile His Val Ala Arg Phe Gly Asn Ile Gly Ser Leu Ser Val Lys Glu Met
2205 2210 2215 2220
Ser Ser Asn Gln Lys Ser Pro Thr Lys Thr Ser Lys Ser Pro Gly Thr Ala Asn Val Leu
2225 2230 2235 2240
Asp Val Asn Asn Ser Thr Leu Met Phe Val Gly Gly Leu Gly Gly Gln Ile Lys Lys Ser
2245 2250 2255 2260
Pro Ala Val Lys Val Thr His Phe Lys Gly Cys Leu Gly Glu Ala Phe Leu Asn Gly Lys
2265 2270 2275 2280
Ser Ile Gly Leu Trp Asn Tyr Ile Glu Arg Glu Gly Lys Cys Arg Gly Cys Phe Gly Ser
2285 2290 2295 2300
Ser Gln Asn Glu Asp Pro Ser Phe His Phe Asp Gly Ser Gly Tyr Ser Val Val Glu Lys
2305 2310 2315 2320
Ser Leu Pro Ala Thr Val Thr Gln Ile Ile Met Leu Phe Asn Thr Phe Ser Pro Asn Gly
2325 2330 2335 2340
Leu Leu Leu Tyr Leu Gly Ser Tyr Gly Thr Lys Asp Phe Leu Ser Ile Glu Leu Phe Arg
2345 2350 2355 2360
Gly Arg Val Lys Val Met Thr Asp Leu Gly Ser Gly Pro Ile Thr Leu Leu Thr Asp Arg
2365 2370 2375 2380
Arg Tyr Asn Asn Gly Thr Trp Tyr Lys Ile Ala Phe Gln Arg Asn Arg Lys Gln Gly Val
2385 2390 2395 2400
Leu Ala Val Ile Asp Ala Tyr Asn Thr Ser Asn Lys Glu Thr Lys Gln Gly Glu Thr Pro
2405 2410 2415 2420
Gly Ala Ser Ser Asp Leu Asn Arg Leu Asp Lys Asp Pro Ile Tyr Val Gly Gly Leu Pro
2425 2430 2435 2440
Arg Ser Arg Val Val Arg Arg Gly Val Thr Thr Lys Ser Phe Val Gly Cys Ile Lys Asn
2445 2450 2455 2460
Leu Glu Ile Ser Arg Ser Thr Phe Asp Leu Leu Arg Asn Ser Tyr Gly Val Arg Lys Gly
2465 2470 2475 2480
Cys Leu Leu Glu Pro Ile Arg Ser Val Ser Phe Leu Lys Gly Gly Tyr Ile Glu Leu Pro
2485 2490 2495 2500
Pro Lys Ser Leu Ser Pro Glu Ser Glu Trp Leu Val Thr Phe Ala Thr Thr Asn Ser Ser
2505 2510 2515 2520
Gly Ile Ile Leu Ala Ala Leu Gly Gly Asp Val Glu Lys Arg Gly Asp Arg Glu Glu Ala
2525 2530 2535 2540
His Val Pro Phe Phe Ser Val Met Leu Ile Gly Gly Asn Ile Glu Val His Val Asn Pro
2545 2550 2555 2560
Gly Asp Gly Thr Gly Leu Arg Lys Ala Leu Leu His Ala Pro Thr Gly Thr Cys Ser Asp
2565 2570 2575 2580
Gly Gln Ala His Ser Ile Ser Leu Val Arg Asn Arg Arg Ile Ile Thr Val Gln Leu Asp
2585 2590 2595 2600
Glu Asn Asn Pro Val Glu Met Lys Leu Gly Thr Leu Val Glu Ser Arg Thr Ile Asn Val
2605 2610 2615 2620
Ser Asn Leu Tyr Val Gly Gly Ile Pro Glu Gly Glu Gly Thr Ser Leu Leu Thr Met Arg
2625 2630 2635 2640
Arg Ser Phe His Gly Cys Ile Lys Asn Leu Ile Phe Asn Leu Glu Leu Leu Asp Phe Asn
2645 2650 2655 2660
Ser Ala Val Gly His Glu Gln Val Asp Leu Asp Thr Cys Trp Leu Ser Glu Arg Pro Lys
2665 2670 2675 2680
Leu Ala Pro Asp Ala Glu Asp Ser Lys Leu Leu Arg Glu Pro Arg Ala Phe Pro Glu Gln
2685 2690 2695 2700
Cys Val Val Asp Ala Ala Leu Glu Tyr Val Pro Gly Ala His Gln Phe Gly Leu Thr Gln
2705 2710 2715 2720
Asn Ser His Phe Ile Leu Pro Phe Asn Gln Ser Ala Val Arg Lys Lys Leu Ser Val Glu
2725 2730 2735 2740
Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu Ile Tyr Tyr Met Ala His Gln Asn Gln
2745 2750 2755 2760
Ala Asp Tyr Ala Val Leu Gln Leu His Gly Gly Arg Leu His Phe Met Phe Asp Leu Gly
2765 2770 2775 2780
Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu Leu Ser Asp Gly Lys Trp His Thr Val
2785 2790 2795 2800
Lys Thr Asp Tyr Val Lys Arg Lys Gly Phe Ile Thr Val Asp Gly Arg Glu Ser Pro Met

```
                2805                2810                2815                2820
Val Thr Val Val Gly Asp Gly Thr Met Leu Asp Val Glu Gly Leu Phe Tyr Leu Gly Gly
                2825                2830                2835                2840
Leu Pro Ser Gln Tyr Gln Ala Arg Lys Ile Gly Asn Ile Thr His Ser Ile Pro Ala Cys
                2845                2850                2855                2860
Ile Gly Asp Val Thr Val Asn Ser Lys Gln Leu Asp Lys Asp Ser Pro Val Ser Ala Phe
                2865                2870                2875                2880
Thr Val Asn Arg Cys Tyr Ala Val Ala Gln Glu Gly Thr Tyr Phe Asp Gly Ser Gly Tyr
                2885                2890                2895                2900
Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Gln Ser Asp Val Asn Ile Thr Leu Glu Phe
                2905                2910                2915                2920
Arg Thr Ser Ser Gln Asn Gly Val Leu Leu Gly Ile Ser Thr Ala Lys Val Asp Ala Ile
                2925                2930                2935                2940
Gly Leu Glu Leu Val Asp Gly Lys Val Leu Phe His Val Asn Asn Gly Ala Gly Arg Ile
                2945                2950                2955                2960
Thr Pro Ala Tyr Glu Pro Lys Thr Ala Thr Val Leu Cys Asp Gly Lys Trp His Thr Leu
                2965                2970                2975                2980
Gln Ala Asn Lys Ser Lys His Arg Ile Thr Leu Ile Val Asp Gly Asn Ala Val Gly Ala
                2985                2990                2995                3000
Glu Ser Pro His Thr Gln Ser Thr Ser Val Asp Thr Asn Asn Pro Ile Tyr Val Gly Gly
                3005                3010                3015                3020
Tyr Pro Ala Gly Val Lys Gln Lys Cys Leu Arg Ser Gln Thr Ser Phe Arg Gly Cys Leu
                3025                3030                3035                3040
Arg Lys Leu Ala Leu Ile Lys Ser Pro Gln Val Gln Ser Phe Asp Phe Ser Arg Ala Phe
                3045                3050                3055                3060
Glu Leu His Gly Val Phe Leu His Ser Cys Pro Gly Thr Glu Ser
                3065                3070                3075
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1786 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P07942;

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Gly Leu Leu Gln Leu Leu Ala Phe Ser Phe Leu Ala Leu Cys Arg Ala Arg Val Arg
1               5                  10                  15                  20
Ala Gln Glu Pro Glu Phe Ser Tyr Gly Cys Ala Glu Gly Ser Cys Tyr Pro Ala Thr Gly
                25                  30                  35                  40
Asp Leu Leu Ile Gly Arg Ala Gln Lys Leu Ser Val Thr Ser Thr Cys Gly Leu His Lys
                45                  50                  55                  60
Pro Glu Pro Tyr Cys Ile Val Ser His Leu Gln Glu Asp Lys Lys Cys Phe Ile Cys Asn
                65                  70                  75                  80
Ser Gln Asp Pro Tyr His Glu Thr Leu Asn Pro Asp Ser His Leu Ile Glu Asn Val Val
                85                  90                  95                 100
Thr Thr Phe Ala Pro Asn Arg Leu Lys Ile Trp Trp Gln Ser Glu Asn Gly Val Glu Asn
                105                 110                 115                 120
Val Thr Ile Gln Leu Asp Leu Glu Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe
                125                 130                 135                 140
Lys Thr Phe Arg Pro Ala Ala Met Leu Ile Glu Arg Ser Ser Asp Phe Gly Lys Thr Trp
                145                 150                 155                 160
Gly Val Tyr Arg Tyr Phe Ala Tyr Asp Cys Glu Ala Ser Phe Pro Gly Ile Ser Thr Gly
```

```
                 165                 170                 175                 180
Pro Met Lys Lys Val Asp Asp Ile Ile Cys Asp Ser Arg Tyr Ser Asp Ile Glu Pro Ser
                 185                 190                 195                 200
Thr Glu Gly Glu Val Ile Phe Arg Ala Leu Asp Pro Ala Phe Lys Ile Glu Asp Pro Tyr
                 205                 210                 215                 220
Ser Pro Arg Ile Gln Asn Leu Leu Lys Ile Thr Asn Leu Arg Ile Lys Phe Val Lys Leu
                 225                 230                 235                 240
His Thr Leu Gly Asp Asn Leu Leu Asp Ser Arg Met Glu Ile Arg Glu Lys Tyr Tyr Tyr
                 245                 250                 255                 260
Ala Val Tyr Asp Met Val Val Arg Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Glu Cys
                 265                 270                 275                 280
Ala Pro Val Asp Gly Phe Asn Glu Glu Val Glu Gly Met Val His Gly His Cys Met Cys
                 285                 290                 295                 300
Arg His Asn Thr Lys Gly Leu Asn Cys Glu Leu Cys Met Asp Phe Tyr His Asp Leu Pro
                 305                 310                 315                 320
Trp Arg Pro Ala Glu Gly Arg Asn Ser Asn Ala Cys Lys Lys Cys Asn Cys Asn Glu His
                 325                 330                 335                 340
Ser Ile Ser Cys His Phe Asp Met Ala Val Tyr Leu Ala Thr Gly Asn Val Ser Gly Gly
                 345                 350                 355                 360
Val Cys Asp Asp Cys Gln His Asn Thr Met Gly Arg Asn Cys Glu Gln Cys Lys Pro Phe
                 365                 370                 375                 380
Tyr Tyr Gln His Pro Glu Arg Asp Ile Arg Asp Pro Asn Phe Cys Glu Arg Cys Thr Cys
                 385                 390                 395                 400
Asp Pro Ala Gly Ser Gln Asn Glu Gly Ile Cys Asp Ser Tyr Thr Asp Phe Ser Thr Gly
                 405                 410                 415                 420
Leu Ile Ala Gly Gln Cys Arg Cys Lys Leu Asn Val Glu Gly Glu His Cys Asp Val Cys
                 425                 430                 435                 440
Lys Glu Gly Phe Tyr Asp Leu Ser Ser Glu Asp Pro Phe Gly Cys Lys Ser Cys Ala Cys
                 445                 450                 455                 460
Asn Pro Leu Gly Thr Ile Pro Gly Gly Asn Pro Cys Asp Ser Glu Thr Gly His Cys Tyr
                 465                 470                 475                 480
Cys Lys Arg Leu Val Thr Gly Gln His Cys Asp Gln Cys Leu Pro Glu His Trp Gly Leu
                 485                 490                 495                 500
Ser Asn Asp Leu Asp Gly Cys Arg Pro Cys Asp Cys Asp Leu Gly Gly Ala Leu Asn Asn
                 505                 510                 515                 520
Ser Cys Phe Ala Glu Ser Gly Gln Cys Ser Cys Arg Pro His Met Ile Gly Arg Gln Cys
                 525                 530                 535                 540
Asn Glu Val Glu Pro Gly Tyr Tyr Phe Ala Thr Leu Asp His Tyr Leu Tyr Glu Ala Glu
                 545                 550                 555                 560
Glu Ala Asn Leu Gly Pro Gly Val Ser Ile Val Glu Arg Gln Tyr Ile Gln Asp Arg Ile
                 565                 570                 575                 580
Pro Ser Trp Thr Gly Ala Gly Phe Val Arg Val Pro Glu Gly Ala Tyr Leu Glu Phe Phe
                 585                 590                 595                 600
Ile Asp Asn Ile Pro Tyr Ser Met Glu Tyr Asp Ile Leu Ile Arg Tyr Glu Pro Gln Leu
                 605                 610                 615                 620
Pro Asp His Trp Glu Lys Ala Val Ile Thr Val Gln Arg Pro Gly Arg Ile Pro Thr Ser
                 625                 630                 635                 640
Ser Arg Cys Gly Asn Thr Ile Pro Asp Asp Asp Asn Gln Val Val Ser Leu Ser Pro Gly
                 645                 650                 655                 660
Ser Arg Tyr Val Val Leu Pro Arg Pro Val Cys Phe Glu Lys Gly Thr Asn Tyr Thr Val
                 665                 670                 675                 680
Arg Leu Glu Leu Pro Gln Tyr Thr Ser Ser Asp Ser Asp Val Glu Ser Pro Tyr Thr Leu
                 685                 690                 695                 700
Ile Asp Ser Leu Val Leu Met Pro Tyr Cys Lys Ser Leu Asp Ile Phe Thr Val Gly Gly
                 705                 710                 715                 720
Ser Gly Asp Gly Val Val Thr Asn Ser Ala Trp Glu Thr Phe Gln Arg Tyr Arg Cys Leu
                 725                 730                 735                 740
Glu Asn Ser Arg Ser Val Val Lys Thr Pro Met Thr Asp Val Cys Arg Asn Ile Ile Phe
                 745                 750                 755                 760
Ser Ile Ser Ala Leu Leu His Gln Thr Gly Leu Ala Cys Glu Cys Asp Pro Gln Gly Ser
                 765                 770                 775                 780
Leu Ser Ser Val Cys Asp Pro Asn Gly Gly Gln Cys Gln Cys Arg Pro Asn Val Val Gly
                 785                 790                 795                 800
```

Arg Thr Cys Asn Arg Cys Ala Pro Gly Thr Phe Gly Phe Gly Pro Ser Gly Cys Lys Pro
805 810 815 820
Cys Glu Cys His Leu Gln Gly Ser Val Asn Ala Phe Cys Asn Pro Val Thr Gly Gln Cys
825 830 835 840
His Cys Ph Gln Gly Val Tyr Ala Arg Gln Cys Asp Arg Cys Leu Pro Gly His Trp Gly
845 850 855 860
Phe Pro Ser Cys Gln Pro Cys Gln Cys Asn Gly His Ala Asp Asp Cys Asp Pro Val Thr
865 870 875 880
Gly Glu Cys Leu Asn Cys Gln Asp Tyr Thr Met Gly His Asn Cys Glu Arg Cys Leu Ala
885 890 895 900
Gly Tyr Tyr Gly Asp Pro Ile Ile Gly Ser Gly Asp His Cys Arg Pro Cys Pro Cys Pro
905 910 915 920
Asp Gly Pro Asp Ser Gly Arg Gln Phe Ala Arg Ser Cys Tyr Gln Asp Pro Val Thr Leu
925 930 935 940
Gln Leu Ala Cys Val Cys Asp Pro Gly Tyr Ile Gly Ser Arg Cys Asp Asp Cys Ala Ser
945 950 955 960
Gly Tyr Phe Gly Asn Pro Ser Glu Val Gly Gly Ser Cys Gln Pro Cys Gln Cys His Asn
965 970 975 980
Asn Ile Asp Thr Thr Asp Pro Glu Ala Cys Asp Lys Glu Thr Gly Arg Cys Leu Lys Cys
985 990 995 1000
Leu Tyr His Thr Glu Gly Glu His Cys Gln Phe Cys Arg Phe Gly Tyr Tyr Gly Asp Ala
1005 1010 1015 1020
Leu Arg Gln Asp Cys Arg Lys Cys Val Cys Asn Tyr Leu Gly Thr Val Gln Glu His Cys
1025 1030 1035 1040
Asn Gly Ser Asp Cys Gln Cys Asp Lys Ala Thr Gly Gln Cys Leu Cys Leu Pro Asn Val
1045 1050 1055 1060
Ile Gly Gln Asn Cys Asp Arg Cys Ala Pro Asn Thr Trp Gln Leu Ala Ser Gly Thr Gly
1065 1070 1075 1080
Cys Asp Pro Cys Asn Cys Asn Ala Ala His Ser Phe Gly Pro Ser Cys Asn Glu Phe Thr
1085 1090 1095 1100
Gly Gln Cys Gln Cys Met Pro Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu
1105 1110 1115 1120
Phe Trp Gly Asp Pro Asp Val Glu Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Glu
1125 1130 1135 1140
Thr Pro Gln Cys Asp Gln Ser Thr Gly Gln Cys Val Cys Val Glu Gly Val Glu Gly Pro
1145 1150 1155 1160
Arg Cys Asp Lys Cys Thr Arg Gly Tyr Ser Gly Val Phe Pro Asp Cys Thr Pro Cys His
1165 1170 1175 1180
Gln Cys Phe Ala Leu Trp Asp Val Ile Ile Ala Glu Leu Thr Asn Arg Thr His Arg Phe
1185 1190 1195 1200
Leu Glu Lys Ala Lys Ala Leu Lys Ile Ser Gly Val Ile Gly Pro Tyr Arg Glu Thr Val
1205 1210 1215 1220
Asp Ser Val Glu Arg Lys Val Ser Glu Ile Lys Asp Ile Leu Ala Gln Ser Pro Ala Ala
1225 1230 1235 1240
Glu Pro Leu Lys Asn Ile Gly Asn Leu Phe Glu Glu Ala Glu Lys Leu Ile Lys Asp Val
1245 1250 1255 1260
Thr Glu Met Met Ala Gln Val Glu Val Lys Leu Ser Asp Thr Thr Ser Gln Ser Asn Ser
1265 1270 1275 1280
Thr Ala Lys Glu Leu Asp Ser Leu Gln Thr Glu Ala Glu Ser Leu Asp Asn Thr Val Lys
1285 1290 1295 1300
Glu Leu Ala Glu Gln Leu Glu Phe Ile Lys Asn Ser Asp Ile Arg Gly Ala Leu Asp Ser
1305 1310 1315 1320
Ile Thr Lys Tyr Phe Gln Met Ser Leu Glu Ala Glu Glu Arg Val Asn Ala Ser Thr Thr
1325 1330 1335 1340
Glu Pro Asn Ser Thr Val Glu Gln Ser Ala Leu Met Arg Asp Arg Val Glu Asp Val Met
1345 1350 1355 1360
Met Glu Arg Glu Ser Gln Phe Lys Glu Lys Gln Glu Glu Gln Ala Arg Leu Leu Asp Glu
1365 1370 1375 1380
Leu Ala Gly Lys Leu Gln Ser Leu Asp Leu Ser Ala Ala Ala Glu Met Thr Cys Gly Thr
1385 1390 1395 1400
Pro Pro Gly Ala S r Cys Ser Glu Thr Glu Cys Gly Gly Pro Asn Cys Arg Thr Asp Glu
1405 1410 1415 1420
Gly Glu Arg Lys Cys Gly Gly Pro Gly Cys Gly Gly Leu Val Thr Val Ala His Asn Ala

```
                1425                1430                1435                1440
Trp Gln Lys Ala Met Asp Leu Asp Gln Asp Val Leu Ser Ala Leu Ala Glu Val Glu Gln
                1445                1450                1455                1460
Leu Ser Lys Met Val Ser Glu Ala Lys Leu Arg Ala Asp Glu Ala Lys Gln Ser Ala Glu
                1465                1470                1475                1480
Asp Ile Leu Leu Lys Thr Asn Ala Thr Lys Glu Lys Met Asp Lys Ser Asn Glu Glu Leu
                1485                1490                1495                1500
Arg Asn Leu Ile Lys Gln Ile Arg Asn Phe Leu Thr Gln Asp Ser Ala Asp Leu Asp Ser
                1505                1510                1515                1520
Ile Glu Ala Val Ala Asn Glu Val Leu Lys Met Glu Met Pro Ser Thr Pro Gln Gln Leu
                1525                1530                1535                1540
Gln Asn Leu Thr Glu Asp Ile Arg Glu Arg Val Glu Ser Leu Ser Gln Val Glu Val Ile
                1545                1550                1555                1560
Leu Gln His Ser Ala Ala Asp Ile Ala Arg Ala Glu Met Leu Leu Glu Glu Ala Lys Arg
                1565                1570                1575                1580
Ala Ser Lys Ser Ala Thr Asp Val Lys Val Thr Ala Asp Met Val Lys Glu Ala Leu Glu
                1585                1590                1595                1600
Glu Ala Glu Lys Ala Gln Val Ala Ala Glu Lys Ala Ile Lys Gln Ala Asp Glu Asp Ile
                1605                1610                1615                1620
Gln Gly Thr Gln Asn Leu Leu Thr Ser Ile Glu Ser Glu Thr Ala Ala Ser Glu Glu Thr
                1625                1630                1635                1640
Leu Phe Asn Ala Ser Gln Arg Ile Ser Glu Leu Glu Arg Asn Val Glu Glu Leu Lys Arg
                1645                1650                1655                1660
Lys Ala Ala Gln Asn Ser Gly Glu Ala Glu Tyr Ile Glu Lys Val Val Tyr Thr Val Lys
                1665                1670                1675                1680
Gln Ser Ala Glu Asp Val Lys Lys Thr Leu Asp Gly Glu Leu Asp Glu Lys Tyr Lys Lys
                1685                1690                1695                1700
Val Glu Asn Leu Ile Ala Lys Lys Thr Glu Glu Ser Ala Asp Ala Arg Arg Lys Ala Glu
                1705                1710                1715                1720
Met Leu Gln Asn Glu Ala Lys Thr Leu Leu Ala Gln Ala Asn Ser Lys Leu Gln Leu Leu
                1725                1730                1735                1740
Lys Asp Leu Glu Arg Lys Tyr Glu Asp Asn Gln Arg Tyr Leu Glu Asp Lys Ala Gln Glu
                1745                1750                1755                1760
Leu Ala Arg Leu Glu Gly Glu Val Arg Ser Leu Leu Lys Asp Ile Ser Gln Lys Val Ala
                1765                1770                1775                1780
Val Tyr Ser Thr Cys Leu
                1785
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1786 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P02469

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Gly Leu Leu Gln Val Phe Ala Phe Gly Val Leu Ala Leu Trp Gly Thr Arg Val Cys
1               5                   10                  15                  20
Ala Gln Glu Pro Glu Phe Ser Tyr Gly Cys Ala Glu Gly Ser Cys Tyr Pro Ala Thr Gly
                25                  30                  35                  40
Asp Leu L u Ile Gly Arg Ala Gln Lys Leu Ser Val Thr Ser Thr Cys Gly Leu His Lys
                45                  50                  55                  60
Pro Glu Pro Tyr Cys Ile Val Ser His Leu Gln Glu Asp Lys Lys Cys Phe Ile Cys Asp
```

65 70 75 80
S r Arg Asp Pro Tyr His Glu Thr Leu Asn Pro Asp Ser His Leu Ile Glu Asn Val Val
85 90 95 100
Thr Thr Phe Ala Pro Asn Arg Leu Lys Ile Trp Trp Gln Ser Glu Asn Gly Val Glu Asn
105 110 115 120
Val Thr Ile Gln Leu Asp Leu Glu Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe
125 130 135 140
Lys Thr Phe Arg Pro Ala Ala Met Leu Ile Glu Arg Ser Ser Asp Phe Gly Lys Thr Trp
145 150 155 160
Gly Val Tyr Arg Tyr Phe Ala Tyr Asp Cys Glu Ser Ser Phe Pro Gly Ile Ser Thr Gly
165 170 175 180
Pro Met Lys Lys Val Asp Asp Ile Ile Cys Asp Ser Arg Tyr Ser Asp Ile Glu Pro Ser
185 190 195 200
Thr Glu Gly Glu Val Ile Phe Arg Ala Leu Asp Pro Ala Phe Lys Ile Glu Asp Pro Tyr
205 210 215 220
Ser Pro Arg Ile Gln Asn Leu Leu Lys Ile Thr Asn Leu Arg Ile Lys Phe Val Lys Leu
225 230 235 240
His Thr Leu Gly Asp Asn Leu Leu Asp Ser Arg Met Glu Ile Arg Glu Lys Tyr Tyr Tyr
245 250 255 260
Ala Val Tyr Asp Met Val Val Arg Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Glu Cys
265 270 275 280
Ala Pro Val Asp Gly Val Asn Glu Glu Val Glu Gly Met Val His Gly His Cys Met Cys
285 290 295 300
Arg His Asn Thr Lys Gly Leu Asn Cys Glu Leu Cys Met Asp Phe Tyr His Asp Leu Pro
305 310 315 320
Trp Arg Pro Ala Glu Gly Arg Asn Ser Asn Ala Cys Lys Lys Cys Asn Cys Asn Glu His
325 330 335 340
Ser Ser Ser Cys His Phe Asp Met Ala Val Phe Leu Ala Thr Gly Asn Val Ser Gly Gly
345 350 355 360
Val Cys Asp Asn Cys Gln His Asn Thr Met Gly Arg Asn Cys Glu Gln Cys Lys Pro Phe
365 370 375 380
Tyr Phe Gln His Pro Glu Arg Asp Ile Arg Asp Pro Asn Leu Cys Glu Pro Cys Thr Cys
385 390 395 400
Asp Pro Ala Gly Ser Glu Asn Gly Gly Ile Cys Asp Gly Tyr Thr Asp Phe Ser Val Gly
405 410 415 420
Leu Ile Ala Gly Gln Cys Arg Cys Lys Leu His Val Glu Gly Glu Arg Cys Asp Val Cys
425 430 435 440
Lys Glu Gly Phe Tyr Asp Leu Ser Ala Glu Asp Pro Tyr Gly Cys Lys Ser Cys Ala Cys
445 450 455 460
Asn Pro Leu Gly Thr Ile Pro Gly Gly Asn Pro Cys Asp Ser Glu Thr Gly Tyr Cys Tyr
465 470 475 480
Cys Lys Arg Leu Val Thr Gly Gln Arg Cys Asp Gln Cys Leu Pro Gln His Trp Gly Leu
485 490 495 500
Ser Asn Asp Leu Asp Gly Cys Arg Pro Cys Asp Cys Asp Leu Gly Gly Ala Leu Asn Asn
505 510 515 520
Ser Cys Ser Glu Asp Ser Gly Gln Cys Ser Cys Leu Pro His Met Ile Gly Arg Gln Cys
525 530 535 540
Asn Glu Val Glu Ser Gly Tyr Tyr Phe Thr Thr Leu Asp His Tyr Ile Tyr Glu Ala Glu
545 550 555 560
Glu Ala Asn Leu Gly Pro Gly Val Val Val Val Glu Arg Gln Tyr Ile Gln Asp Arg Ile
565 570 575 580
Pro Ser Trp Thr Gly Pro Gly Phe Val Arg Val Pro Glu Gly Ala Tyr Leu Glu Phe Phe
585 590 595 600
Ile Asp Asn Ile Pro Tyr Ser Met Glu Tyr Glu Ile Leu Ile Arg Tyr Glu Pro Gln Leu
605 610 615 620
Pro Asp His Trp Glu Lys Ala Val Ile Thr Val Gln Arg Pro Gly Lys Ile Pro Ala Ser
625 630 635 640
Ser Arg Cys Gly Asn Thr Val Pro Asp Asp Asp Asn Gln Val Val Ser Leu Ser Pro Gly
645 650 655 660
Ser Arg Tyr Val Val Leu Pro Arg Pro Val Cys Phe Glu Lys Gly Met Asn Tyr Thr Val
665 670 675 680
Arg Leu Glu Leu Pro Gln Tyr Thr Ala Ser Gly Ser Asp Val Glu Ser Pro Tyr Thr Phe
685 690 695 700

Ile Asp Ser Leu Val Leu Met Pro Tyr Cys Lys Ser Leu Asp Ile Phe Thr Val Gly Gly
705 710 715 720
Ser Gly Asp Gly Glu Val Thr Asn Ser Ala Trp Glu Thr Phe Gln Arg Tyr Arg Cys Leu
725 730 735 740
Glu Asn Ser Arg Ser Val Val Lys Thr Pro Met Thr Asp Val Cys Arg Asn Ile Ile Phe
745 750 755 760
Ser Ile Ser Ala Leu Ile His Gln Thr Gly Leu Ala Cys Glu Cys Asp Pro Gln Gly Ser
765 770 775 780
Leu Ser Ser Val Cys Asp Pro Asn Gly Gly Gln Cys Gln Cys Arg Pro Asn Val Val Gly
785 790 795 800
Arg Thr Cys Asn Arg Cys Ala Pro Gly Thr Phe Gly Phe Gly Pro Asn Gly Cys Lys Pro
805 810 815 820
Cys Asp Cys His Leu Gln Gly Ser Ala Ser Ala Phe Cys Asp Ala Ile Thr Gly Gln Cys
825 830 835 840
His Cys Phe Gln Gly Ile Tyr Ala Arg Gln Cys Asp Arg Cys Leu Pro Gly Tyr Trp Gly
845 850 855 860
Phe Pro Ser Cys Gln Pro Cys Gln Cys Asn Gly His Ala Leu Asp Cys Asp Thr Val Thr
865 870 875 880
Gly Glu Cys Leu Ser Cys Gln Asp Tyr Thr Thr Gly His Asn Cys Glu Arg Cys Leu Ala
885 890 895 900
Gly Tyr Tyr Gly Asp Pro Ile Ile Gly Ser Gly Asp His Cys Arg Pro Cys Pro Cys Pro
905 910 915 920
Asp Gly Pro Asp Ser Gly Arg Gln Phe Ala Arg Ser Cys Tyr Gln Asp Pro Val Thr Leu
925 930 935 940
Gln Leu Ala Cys Val Cys Asp Pro Gly Tyr Ile Gly Ser Arg Cys Asp Asp Cys Ala Ser
945 950 955 960
Gly Phe Phe Gly Asn Pro Ser Asp Phe Gly Gly Ser Cys Gln Pro Cys Gln Cys His His
965 970 975 980
Asn Ile Asp Thr Thr Asp Pro Glu Ala Cys Asp Lys Asp Thr Gly Arg Cys Leu Lys Cys
985 990 995 1000
Leu Tyr His Thr Glu Gly Asp His Cys Gln Leu Cys Gln Tyr Gly Tyr Tyr Gly Asp Ala
1005 1010 1015 1020
Leu Arg Gln Asp Cys Arg Lys Cys Val Cys Asn Tyr Leu Gly Thr Val Lys Glu His Cys
1025 1030 1035 1040
Asn Gly Ser Asp Cys His Cys Asp Lys Ala Thr Gly Gln Cys Ser Cys Leu Pro Asn Val
1045 1050 1055 1060
Ile Gly Gln Asn Cys Asp Arg Cys Ala Pro Asn Thr Trp Gln Leu Ala Ser Gly Thr Gly
1065 1070 1075 1080
Cys Gly Pro Cys Asn Cys Asn Ala Ala His Ser Phe Gly Pro Ser Cys Asn Glu Phe Thr
1085 1090 1095 1100
Gly Gln Cys Gln Cys Met Pro Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu
1105 1110 1115 1120
Phe Trp Gly Asp Pro Asp Val Glu Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Glu
1125 1130 1135 1140
Thr Pro Gln Cys Asp Gln Ser Thr Gly Gln Cys Val Cys Val Glu Gly Val Glu Gly Pro
1145 1150 1155 1160
Arg Cys Asp Lys Cys Thr Arg Gly Tyr Ser Gly Val Phe Pro Asp Cys Thr Pro Cys His
1165 1170 1175 1180
Gln Cys Phe Ala Leu Trp Asp Ala Ile Ile Gly Glu Leu Thr Asn Arg Thr His Lys Phe
1185 1190 1195 1200
Leu Glu Lys Ala Lys Ala Leu Lys Ile Ser Gly Val Ile Gly Pro Tyr Arg Glu Thr Val
1205 1210 1215 1220
Asp Ser Val Glu Lys Lys Val Asn Glu Ile Lys Asp Ile Leu Ala Gln Ser Pro Ala Ala
1225 1230 1235 1240
Glu Pro Leu Lys Asn Ile Gly Ile Leu Phe Glu Glu Ala Glu Lys Leu Thr Lys Asp Val
1245 1250 1255 1260
Thr Glu Lys Met Ala Gln Val Glu Val Lys Leu Thr Asp Thr Ala Ser Gln Ser Asn Ser
1265 1270 1275 1280
Thr Ala Gly Glu Leu Gly Ala Leu Gln Ala Glu Ala Glu Ser Leu Asp Lys Thr Val Lys
1285 1290 1295 1300
Glu Leu Ala Glu Gln Leu Glu Phe Ile Lys Asn Ser Asp Ile Gln Gly Ala Leu Asp Ser
1305 1310 1315 1320
Ile Thr Lys Tyr Phe Gln Met Ser Leu Glu Ala Glu Lys Arg Val Asn Ala Ser Thr Thr

```
                1325                1330                1335                1340
Asp Pro Asn Ser Thr Val Glu Gln Ser Ala Leu Thr Arg Asp Arg Val Glu Asp Leu Met
                1345                1350                1355                1360
Leu Glu Arg Glu Ser Pro Phe Lys Glu Gln Gln Glu Glu Gln Ala Arg Leu Leu Asp Glu
                1365                1370                1375                1380
Leu Ala Gly Lys Leu Gln Ser Leu Asp Leu Ser Ala Ala Ala Gln Met Thr Cys Gly Thr
                1385                1390                1395                1400
Pro Pro Gly Ala Asp Cys Ser Glu Ser Glu Cys Gly Gly Pro Asn Cys Arg Thr Asp Glu
                1405                1410                1415                1420
Gly Glu Lys Lys Cys Gly Gly Pro Gly Cys Gly Gly Leu Val Thr Val Ala His Ser Ala
                1425                1430                1435                1440
Trp Gln Lys Ala Met Asp Phe Asp Arg Asp Val Leu Ser Ala Leu Ala Glu Val Glu Gln
                1445                1450                1455                1460
Leu Ser Lys Met Val Ser Glu Ala Lys Val Arg Ala Asp Glu Ala Lys Gln Asn Ala Gln
                1465                1470                1475                1480
Asp Val Leu Leu Lys Thr Asn Ala Thr Lys Glu Lys Val Asp Lys Ser Asn Glu Asp Leu
                1485                1490                1495                1500
Arg Asn Leu Ile Lys Gln Ile Arg Asn Phe Leu Thr Glu Asp Ser Ala Asp Leu Asp Ser
                1505                1510                1515                1520
Ile Glu Ala Val Ala Asn Glu Val Leu Lys Ser Gly Asn Ala Ser Thr Pro Gln Gln Leu
                1525                1530                1535                1540
Gln Asn Leu Thr Glu Asp Ile Arg Glu Arg Val Glu Thr Leu Ser Gln Val Glu Val Ile
                1545                1550                1555                1560
Leu Gln Gln Ser Ala Ala Asp Ile Ala Arg Ala Glu Leu Leu Leu Glu Glu Ala Lys Arg
                1565                1570                1575                1580
Ala Ser Lys Ser Ala Thr Asp Val Lys Val Thr Ala Asp Met Val Lys Glu Ala Leu Glu
                1585                1590                1595                1600
Glu Ala Glu Lys Ala Gln Val Ala Ala Glu Lys Ala Ile Lys Gln Ala Asp Glu Asp Ile
                1605                1610                1615                1620
Gln Gly Thr Gln Asn Leu Leu Thr Ser Ile Glu Ser Glu Thr Ala Ala Ser Glu Glu Thr
                1625                1630                1635                1640
Leu Thr Asn Ala Ser Gln Arg Ile Ser Lys Leu Glu Arg Asn Val Glu Glu Leu Lys Arg
                1645                1650                1655                1660
Lys Ala Ala Gln Asn Ser Gly Glu Ala Glu Tyr Ile Glu Lys Val Val Tyr Ser Val Lys
                1665                1670                1675                1680
Gln Asn Ala Asp Asp Val Lys Lys Thr Leu Asp Gly Glu Leu Asp Glu Lys Tyr Lys Lys
                1685                1690                1695                1700
Val Glu Ser Leu Ile Ala Gln Lys Thr Glu Glu Ser Ala Asp Ala Arg Arg Lys Ala Glu
                1705                1710                1715                1720
Leu Leu Gln Asn Glu Ala Lys Thr Leu Leu Ala Gln Ala Asn Ser Lys Leu Gln Leu Leu
                1725                1730                1735                1740
Glu Asp Leu Glu Arg Lys Tyr Glu Asp Asn Gln Lys Tyr Leu Glu Asp Lys Ala Gln Glu
                1745                1750                1755                1760
Leu Val Arg Leu Glu Gly Glu Val Arg Ser Leu Leu Lys Asp Ile Ser Glu Lys Val Ala
                1765                1770                1775                1780
Val Tyr Ser Thr Cys Leu
                1785
```

(2)INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1801 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P15800

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Glu Trp Ala Ser Gly Lys Pro Gly Arg Gly Arg Gln Gly Gln Pro Val Pro Trp Glu
1               5                  10                  15                  20
Leu Arg Leu Gly Leu Leu Leu Ser Val Leu Ala Ala Thr Leu Ala Gln Val Pro Ser Leu
                25                  30                  35                  40
Asp Val Pro Gly Cys Ser Arg Gly Ser Cys Tyr Pro Ala Thr Gly Asp Leu Leu Val Gly
                45                  50                  55                  60
Arg Ala Asp Arg Leu Thr Ala Ser Ser Thr Cys Gly Leu His Ser Pro Gln Pro Tyr Cys
                65                  70                  75                  80
Ile Val Ser His Leu Gln Asp Glu Lys Lys Cys Phe Leu Cys Asp Ser Arg Arg Pro Phe
                85                  90                  95                 100
S r Ala Arg Asp Asn Pro Asn Ser His Arg Ile Gln Asn Val Val Thr Ser Phe Ala Pro
               105                 110                 115                 120
Gln Arg Arg Thr Ala Trp Trp Gln Ser Glu Asn Gly Val Pro Met Val Thr Ile Gln Leu
               125                 130                 135                 140
Asp Leu Glu Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe Lys Thr Phe Arg Pro
               145                 150                 155                 160
Ala Ala Met Leu Val Glu Arg Ser Ala Asp Phe Gly Arg Thr Trp Arg Val Tyr Arg Tyr
               165                 170                 175                 180
Phe Ser Tyr Asp Cys Gly Ala Asp Phe Pro Gly Ile Pro Leu Ala Pro Pro Arg Arg Trp
               185                 190                 195                 200
Asp Asp Val Val Cys Glu Ser Arg Tyr Ser Glu Ile Glu Pro Ser Thr Glu Gly Glu Val
               205                 210                 215                 220
Ile Tyr Arg Val Leu Asp Pro Ala Ile Pro Ile Pro Asp Pro Tyr Ser Ser Arg Ile Gln
               225                 230                 235                 240
Asn Leu Leu Lys Ile Thr Asn Leu Arg Val Asn Leu Thr Arg Leu His Thr Leu Gly Asp
               245                 250                 255                 260
Asn Leu Leu Asp Pro Arg Arg Glu Ile Arg Glu Lys Tyr Tyr Tyr Ala Leu Tyr Glu Leu
               265                 270                 275                 280
Val Ile Arg Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Gln Cys Ala Pro Ala Pro Gly
               285                 290                 295                 300
Ala Pro Ala His Ala Glu Gly Met Val His Gly Ala Cys Ile Cys Lys His Asn Thr Arg
               305                 310                 315                 320
Gly Leu Asn Cys Glu Gln Cys Gln Asp Phe Tyr Gln Asp Leu Pro Trp His Pro Ala Glu
               325                 330                 335                 340
Asp Gly His Thr His Ala Cys Arg Lys Cys Glu Cys Asn Gly His Ser His Ser Cys His
               345                 350                 355                 360
Phe Asp Met Ala Val Tyr Leu Ala Ser Gly Asn Val Ser Gly Gly Val Cys Asp Gly Cys
               365                 370                 375                 380
Gln His Asn Thr Ala Gly Arg His Cys Glu Leu Cys Arg Pro Phe Phe Tyr Arg Asp Pro
               385                 390                 395                 400
Thr Lys Asp Met Arg Asp Pro Ala Ala Cys Arg Pro Cys Asp Cys Asp Pro Met Gly Ser
               405                 410                 415                 420
Gln Asp Gly Gly Arg Cys Asp Ser His Asp Asp Pro Val Leu Gly Leu Val Ser Gly Gln
               425                 430                 435                 440
Cys Arg Cys Lys Glu His Val Val Gly Thr Arg Cys Gln Gln Cys Arg Asp Gly Phe Phe
               445                 450                 455                 460
Gly Leu Ser Ala Ser Asn Pro Arg Gly Cys Gln Arg Cys Gln Cys Asn Ser Arg Gly Thr
               465                 470                 475                 480
Val Pro Gly Gly Thr Pro Cys Asp Ser Ser Ser Gly Thr Cys Phe Cys Lys Arg Leu Val
               485                 490                 495                 500
Thr Gly Asp Gly Cys Asp Arg Cys Leu Pro Gly His Trp Gly Leu Ser His Asp Leu Leu
               505                 510                 515                 520
Gly Cys Arg Pro Cys Asp Cys Asp Val Gly Gly Ala Leu Asp Pro Gln Cys Asp Glu Ala
               525                 530                 535                 540
Thr Gly Gln Cys Pro Cys Arg Pro His Met Ile Gly Arg Arg Cys Glu Gln Val Gln Pro
               545                 550                 555                 560
Gly Tyr Phe Arg Pro Phe Leu Asp His Leu Thr Trp Glu Ala Glu Gly Ala His Gly Gln
               565                 570                 575                 580
Val Leu Glu Val Val Glu Arg Leu Val Thr Asn Arg Glu Thr Pro Ser Trp Thr Gly Val
               585                 590                 595                 600
Gly Phe Val Arg Leu Arg Glu Gly Gln Glu Val Glu Phe Leu Val Thr Ser Leu Pro Arg
```

605 610 615 620
Ala Met Asp Tyr Asp Leu Leu Leu Arg Trp Glu Pro Gln Val Pro Glu Gln Trp Ala Glu
625 630 635 640
Leu Glu Leu Val Val Gln Arg Pro Gly Pro Val Ser Ala His Ser Pro Cys Gly His Val
645 650 655 660
Leu Pro Arg Asp Asp Arg Ile Gln Gly Met Leu His Pro Asn Thr Arg Val Leu Val Phe
665 670 675 680
Pro Arg Pro Val Cys Leu Glu Pro Gly Leu Ser Tyr Lys Leu Lys Leu Lys Leu Thr Gly
685 690 695 700
Thr Gly Gly Arg Ala His Pro Glu Thr Pro Tyr Ser Gly Ser Gly Ile Leu Ile Asp Ser
705 710 715 720
Leu Val Leu Gln Pro His Val Leu Met Leu Glu Met Phe Ser Gly Gly Asp Ala Ala Ala
725 730 735 740
Leu Glu Arg Arg Thr Thr Phe Glu Arg Tyr Arg Cys His Glu Glu Gly Leu Met Pro Ser
745 750 755 760
Lys Thr Pro Leu Ser Glu Ala Cys Val Pro Leu Leu Ile Ser Ala Ser Ser Leu Val Tyr
765 770 775 780
Asn Gly Ala Leu Pro Cys Gln Cys Asp Pro Gln Gly Ser Leu Ser Ser Glu Cys Asn Pro
785 790 795 800
His Gly Gly Gln Cys Arg Cys Lys Pro Gly Val Val Gly Arg Arg Cys Asp Ala Cys Ala
805 810 815 820
Thr Gly Tyr Tyr Gly Phe Gly Pro Ala Gly Cys Gln Ala Cys Gln Cys Ser Pro Asp Gly
825 830 835 840
Ala Leu Ser Ala Leu Cys Glu Gly Thr Ser Gly Gln Cys Leu Cys Arg Thr Gly Ala Phe
845 850 855 860
Gly Leu Arg Cys Asp His Cys Gln Arg Gly Gln Trp Gly Phe Pro Asn Cys Arg Pro Cys
865 870 875 880
Val Cys Asn Gly Arg Ala Asp Glu Cys Asp Ala His Thr Gly Ala Cys Leu Gly Cys Arg
885 890 895 900
Asp Tyr Thr Gly Gly Glu His Cys Glu Arg Cys Ile Ala Gly Phe His Gly Asp Pro Arg
905 910 915 920
Leu Pro Tyr Gly Gly Gln Cys Arg Pro Cys Pro Cys Pro Glu Gly Pro Gly Ser Gln Arg
925 930 935 940
His Phe Ala Thr Ser Cys His Arg Asp Gly Tyr Ser Gln Gln Ile Val Cys His Cys Arg
945 950 955 960
Ala Gly Tyr Thr Gly Leu Arg Cys Glu Ala Cys Ala Pro Gly His Phe Gly Asp Pro Ser
965 970 975 980
Lys Pro Gly Gly Arg Cys Gln Leu Cys Glu Cys Ser Gly Asn Ile Asp Pro Thr Asp Pro
985 990 995 1000
Gly Ala Cys Asp Pro His Thr Gly Gln Cys Leu Arg Cys Leu His His Thr Glu Gly Pro
1005 1010 1015 1020
His Cys Gly His Cys Lys Pro Gly Phe His Gly Gln Ala Ala Arg Gln Ser Cys His Arg
1025 1030 1035 1040
Cys Thr Cys Asn Leu Leu Gly Thr Asp Pro Gln Arg Cys Pro Ser Thr Asp Leu Cys His
1045 1050 1055 1060
Cys Asp Pro Ser Thr Gly Gln Cys Pro Cys Leu Pro His Val Gln Gly Leu Ser Cys Asp
1065 1070 1075 1080
Arg Cys Ala Pro Asn Phe Trp Asn Phe Thr Ser Gly Arg Gly Cys Gln Pro Cys Ala Cys
1085 1090 1095 1100
His Pro Ser Arg Ala Arg Gly Pro Thr Cys Asn Glu Phe Thr Gly Gln Cys His Cys His
1105 1110 1115 1120
Ala Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu His Trp Gly Asp Pro Gly
1125 1130 1135 1140
Leu Gln Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Asp Lys Pro Gln Cys His Arg
1145 1150 1155 1160
Ser Thr Gly His Cys Ser Cys Arg Pro Gly Val Ser Gly Val Arg Cys Asp Gln Cys Ala
1165 1170 1175 1180
Arg Gly Phe Ser Gly Val Phe Pro Ala Cys His Pro Cys His Ala Cys Phe Gly Asp Trp
1185 1190 1195 1200
Asp Arg Val Val Gln Asp Leu Ala Ala Arg Thr Arg Arg Leu Glu Gln Trp Ala Gln Glu
1205 1210 1215 1220
Leu Gln Gln Thr Gly Val Leu Gly Ala Phe Glu Ser Ser Phe Leu Asn Leu Gln Gly Lys
1225 1230 1235 1240

Leu Gly Met Val Gln Ala Ile Val Ala Ala Arg Asn Thr Ser Ala Ala Ser Thr Ala Lys
1245 1250 1255 1260
Leu Val Glu Ala Thr Glu Gly Leu Arg His Glu Ile Gly Lys Thr Thr Glu Arg Leu Thr
1265 1270 1275 1280
Gln Leu Glu Ala Glu Leu Thr Asp Val Gln Asp Glu Asn Phe Asn Ala Asn His Ala Leu
1285 1290 1295 1300
Ser Gly Leu Glu Arg Asp Gly Leu Ala Leu Asn Leu Thr Leu Arg Gln Leu Asp Gln His
1305 1310 1315 1320
Leu Asp Ile Leu Lys His Ser Asn Phe Leu Gly Ala Tyr Asp Ser Ile Arg His Ala His
1325 1330 1335 1340
Ser Gln Ser Thr Glu Ala Glu Arg Arg Ala Asn Ala Ser Thr Phe Ala Ile Pro Ser Pro
1345 1350 1355 1360
Val Ser Asn Ser Ala Asp Thr Arg Arg Arg Ala Glu Val Leu Met Gly Ala Gln Arg Glu
1365 1370 1375 1380
Asn Phe Asn Arg Gln His Leu Ala Asn Gln Gln Ala Leu Gly Arg Leu Ser Thr His Thr
1385 1390 1395 1400
His Thr Leu Ser Leu Thr Gly Val Asn Glu Leu Val Cys Gly Ala Pro Gly Asp Ala Pro
1405 1410 1415 1420
Cys Ala Thr Ser Pro Cys Gly Gly Ala Gly Cys Arg Asp Glu Asp Gly Gln Pro Arg Cys
1425 1430 1435 1440
Gly Gly Leu Gly Cys Ser Gly Ala Ala Ala Thr Ala Asp Leu Ala Leu Gly Arg Ala Arg
1445 1450 1455 1460
His Thr Gln Ala Glu Leu Gln Arg Ala Leu Val Glu Gly Gly Gly Ile Leu Ser Arg Val
1465 1470 1475 1480
Ser Glu Thr Arg Arg Gln Ala Glu Glu Ala Gln Gln Arg Ala Gln Ala Ala Leu Asp Lys
1485 1490 1495 1500
Ala Asn Ala Ser Arg Gly Gln Val Glu Gln Ala Asn Gln Glu Leu Arg Glu Leu Ile Gln
1505 1510 1515 1520
Asn Val Lys Asp Phe Leu Ser Gln Glu Gly Ala Asp Pro Asp Ser Ile Glu Met Val Ala
1525 1530 1535 1540
Thr Arg Val Leu Asp Ile Ser Ile Pro Ala Ser Pro Glu Gln Ile Gln Arg Leu Ala Ser
1545 1550 1555 1560
Glu Ile Ala Glu Arg Val Arg Ser Leu Ala Asp Val Asp Thr Ile Leu Ala His Thr Met
1565 1570 1575 1580
Gly Asp Val Arg Arg Ala Glu Gln Leu Leu Gln Asp Ala Gln Arg Ala Arg Ser Arg Ala
1585 1590 1595 1600
Glu Gly Glu Arg Gln Lys Ala Glu Thr Val Gln Ala Ala Leu Glu Glu Ala Gln Arg Ala
1605 1610 1615 1620
Gln Gly Ala Ala Gln Gly Ala Ile Arg Gly Ala Val Val Asp Thr Lys Asn Thr Glu Gln
1625 1630 1635 1640
Thr Leu Gln Gln Val Gln Glu Arg Met Ala Gly Thr Glu Gln Ser Leu Asn Ser Ala Ser
1645 1650 1655 1660
Glu Arg Ala Arg Gln Leu His Ala Leu Leu Glu Ala Leu Lys Leu Lys Arg Ala Gly Asn
1665 1670 1675 1680
Ser Leu Ala Ala Ser Thr Ala Glu Glu Thr Ala Gly Ser Ala Gln Ser Arg Ala Arg Glu
1685 1690 1695 1700
Ala Glu Lys Gln Leu Arg Glu Gln Val Gly Asp Gln Tyr Gln Thr Val Arg Ala Leu Ala
1705 1710 1715 1720
Glu Arg Lys Ala Glu Gly Val Leu Ala Ala Gln Ala Arg Ala Glu Gln Leu Arg Asp Glu
1725 1730 1735 1740
Ala Arg Gly Leu Leu Gln Ala Ala Gln Asp Lys Leu Gln Arg Leu Gln Glu Leu Glu Gly
1745 1750 1755 1760
Thr Tyr Glu Glu Asn Glu Arg Glu Leu Glu Val Lys Ala Ala Gln Leu Asp Gly Leu Glu
1765 1770 1775 1780
Ala Arg Met Arg Ser Val Leu Gln Ala Ile Asn Leu Gln Val Gln Ile Tyr Asn Thr Cys
1785 1790 1795 1800
Gln

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1798 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P55268

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Glu Leu Thr Ser Arg Glu Arg Gly Arg Gly Gln Pro Leu Pro Trp Glu Leu Arg Leu
1               5                  10                  15                  20
Gly Leu Leu Leu Ser Val Leu Ala Ala Thr Leu Ala Gln Ala Pro Ala Pro Asp Val Pro
            25                  30                  35                  40
Gly Cys Ser Arg Gly Ser Cys Tyr Pro Ala Thr Gly Asp Leu Leu Val Gly Arg Ala Asp
        45                  50                  55                  60
Arg Leu Thr Ala Ser Ser Thr Cys Gly Leu Asn Gly Pro Gln Pro Tyr Cys Ile Val Ser
    65                  70                  75                  80
His Leu Gln Asp Glu Lys Lys Cys Phe Leu Cys Asp Ser Arg Arg Pro Phe Ser Ala Arg
                85                  90                  95                  100
Asp Asn Pro His Ser His Arg Ile Gln Asn Val Val Thr Ser Phe Ala Pro Gln Arg Arg
            105                 110                 115                 120
Ala Ala Trp Trp Gln Ser Glu Asn Gly Ile Pro Ala Val Thr Ile Gln Leu Asp Leu Glu
        125                 130                 135                 140
Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe Lys Thr Phe Arg Pro Ala Ala Met
    145                 150                 155                 160
Leu Val Glu Arg Ser Ala Asp Phe Gly Arg Thr Trp His Val Tyr Arg Tyr Phe Ser Tyr
                165                 170                 175                 180
Asp Cys Gly Ala Asp Phe Pro Gly Val Pro Leu Ala Pro Pro Arg His Trp Asp Asp Val
            185                 190                 195                 200
Val Cys Glu Ser Arg Tyr Ser Glu Ile Glu Pro Ser Thr Glu Gly Glu Val Ile Tyr Arg
        205                 210                 215                 220
Val Leu Asp Pro Ala Ile Pro Ile Pro Asp Pro Tyr Ser Ser Arg Ile Gln Asn Leu Leu
    225                 230                 235                 240
Lys Ile Thr Asn Leu Arg Val Asn Leu Thr Arg Leu His Thr Leu Gly Asp Asn Leu Leu
                245                 250                 255                 260
Asp Pro Arg Arg Glu Ile Arg Glu Lys Tyr Tyr Tyr Ala Leu Tyr Glu Leu Val Val Arg
            265                 270                 275                 280
Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Glu Cys Ala Pro Ala Pro Gly Ala Pro Ala
        285                 290                 295                 300
His Ala Glu Gly Met Val His Gly Ala Cys Ile Cys Lys His Asn Thr Arg Gly Leu Asn
    305                 310                 315                 320
Cys Glu Gln Cys Gln Asp Phe Tyr Arg Asp Leu Pro Trp Arg Pro Ala Glu Asp Gly His
                325                 330                 335                 340
Ser His Ala Cys Arg Lys Cys Glu Cys His Gly His Thr His Ser Cys His Phe Asp Met
            345                 350                 355                 360
Ala Val Tyr Leu Ala Ser Gly Asn Val Ser Gly Gly Val Cys Asp Gly Cys Gln His Asn
        365                 370                 375                 380
Thr Ala Gly Arg His Cys Glu Leu Cys Arg Pro Phe Phe Tyr Arg Asp Pro Thr Lys Asp
    385                 390                 395                 400
Leu Arg Asp Pro Ala Val Cys Arg Ser Cys Asp Cys Asp Pro Met Gly Ser Gln Asp Gly
                405                 410                 415                 420
Gly Arg Cys Asp Ser His Asp Asp Pro Ala Leu Gly Leu Val Ser Gly Gln Cys Arg Cys
            425                 430                 435                 440
Lys Glu His Val Val Gly Thr Arg Cys Gln Gln Cys Arg Asp Gly Phe Phe Gly Leu Ser
        445                 450                 455                 460
Ile Ser Asp Arg Leu Gly Cys Arg Arg Cys Gln Cys Asn Ala Arg Gly Thr Val Pro Gly
    465                 470                 475                 480
Ser Thr Pro Cys Asp Pro Asn Ser Gly Ser Cys Tyr Cys Lys Arg Leu Val Thr Gly Arg
                485                 490                 495                 500
```

Gly Cys Asp Arg Cys Leu Pro Gly His Trp Gly Leu Ser His Asp Leu Leu Gly Cys Arg
505 510 515 520
Pro Cys Asp Cys Asp Val Gly Gly Ala Leu Asp Pro Gln Cys Asp Glu Gly Thr Gly Gln
525 530 535 540
Cys His Cys Arg Gln His Met Val Gly Arg Arg Cys Glu Gln Val Gln Pro Gly Tyr Ph
545 550 555 560
Arg Pro Phe Leu Asp His Leu Ile Trp Glu Ala Glu Asp Thr Arg Gly Gln Val Leu Asp
565 570 575 580
Val Val Glu Arg Leu Val Thr Pro Gly Glu Thr Pro Ser Trp Thr Gly Ser Gly Phe Val
585 590 595 600
Arg Leu Gln Glu Gly Gln Thr Leu Glu Phe Leu Val Ala Ser Val Pro Lys Ala Met Asp
605 610 615 620
Tyr Asp Leu Leu Leu Arg Leu Glu Pro Gln Val Pro Glu Gln Trp Ala Glu Leu Glu Leu
625 630 635 640
Ile Val Gln Arg Pro Gly Pro Val Pro Ala His Ser Leu Cys Gly His Leu Val Pro Lys
645 650 655 660
Asp Asp Arg Ile Gln Gly Thr Leu Gln Pro His Ala Arg Tyr Leu Ile Phe Pro Asn Pro
665 670 675 680
Val Cys Leu Glu Pro Gly Ile Ser Tyr Lys Leu His Leu Lys Leu Val Arg Thr Gly Gly
685 690 695 700
Ser Ala Gln Pro Glu Thr Pro Tyr Ser Gly Pro Gly Leu Leu Ile Asp Ser Leu Val Leu
705 710 715 720
Leu Pro Arg Val Leu Val Leu Glu Met Phe Ser Gly Gly Asp Ala Ala Ala Leu Glu Arg
725 730 735 740
Gln Ala Thr Phe Glu Arg Tyr Gln Cys His Glu Glu Gly Leu Val Pro Ser Lys Thr Ser
745 750 755 760
Pro Ser Glu Ala Cys Ala Pro Leu Leu Ile Ser Leu Ser Thr Leu Ile Tyr Asn Gly Ala
765 770 775 780
Leu Pro Cys Gln Cys Asn Pro Gln Gly Ser Leu Ser Ser Glu Cys Asn Pro His Gly Gly
785 790 795 800
Gln Cys Leu Cys Lys Pro Gly Val Val Gly Arg Arg Cys Asp Leu Cys Ala Pro Gly Tyr
805 810 815 820
Tyr Gly Phe Gly Pro Thr Gly Cys Gln Ala Cys Gln Cys Ser His Glu Gly Ala Leu Ser
825 830 835 840
Ser Leu Cys Glu Lys Thr Ser Gly Gln Cys Leu Cys Arg Thr Gly Ala Phe Gly Leu Arg
845 850 855 860
Cys Asp Arg Cys Gln Arg Gly Gln Trp Gly Phe Pro Ser Cys Arg Pro Cys Val Cys Asn
865 870 875 880
Gly His Ala Asp Glu Cys Asn Thr His Thr Gly Ala Cys Leu Gly Cys Arg Asp His Thr
885 890 895 900
Gly Gly Glu His Cys Glu Arg Cys Ile Ala Gly Phe His Arg Asp Pro Arg Leu Pro Tyr
905 910 915 920
Gly Gly Gln Cys Arg Pro Cys Pro Cys Pro Glu Gly Pro Gly Ser Gln Arg His Phe Ala
925 930 935 940
Thr Ser Cys His Gln Asp Glu Tyr Ser Gln Gln Ile Val Cys His Cys Arg Ala Gly Tyr
945 950 955 960
Thr Gly Leu Arg Cys Glu Ala Cys Ala Pro Gly His Phe Gly Asp Pro Ser Arg Pro Gly
965 970 975 980
Gly Arg Cys Gln Leu Cys Glu Cys Ser Gly Asn Ile Asp Pro Met Asp Pro Asp Ala Cys
985 990 995 1000
Asp Pro His Thr Gly Gln Cys Leu Arg Cys Leu His His Thr Glu Gly Pro His Cys Ala
1005 1010 1015 1020
His Cys Lys Pro Gly Phe His Gly Gln Ala Ala Arg Gln Ser Cys His Arg Cys Thr Cys
1025 1030 1035 1040
Asn Leu Leu Gly Thr Asn Pro Gln Gln Cys Pro Ser Pro Asp Gln Cys His Cys Asp Pro
1045 1050 1055 1060
Ser Ser Gly Gln Cys Pro Cys Leu Pro Asn Val Gln Gly Pro Ser Cys Asp Arg Cys Ala
1065 1070 1075 1080
Pro Asn Phe Trp Asn Leu Thr Ser Gly His Gly Cys Gln Pro Cys Ala Cys His Pro Ser
1085 1090 1095 1100
Arg Ala Arg Gly Pro Thr Cys Asn Glu Phe Thr Gly Gln Cys His Cys Arg Ala Gly Phe
1105 1110 1115 1120
Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu His Trp Gly Asp Pro Gly Leu Gln Cys

```
                1125                1130                1135                1140
His Ala Cys Asp Cys Asp Ser Arg Gly Ile Asp Thr Pro Gln Cys His Arg Phe Thr Gly
            1145                1150                1155                1160
His Cys Ser Cys Arg Pro Gly Val Ser Gly Val Arg Cys Asp Gln Cys Ala Arg Gly Phe
            1165                1170                1175                1180
Ser Gly Ile Phe Pro Ala Cys His Pro Cys His Ala Cys Phe Gly Asp Trp Asp Arg Val
            1185                1190                1195                1200
Val Gln Asp Leu Ala Ala Arg Thr Gln Arg Leu Glu Gln Arg Ala Gln Glu Leu Gln Gln
            1205                1210                1215                1220
Thr Gly Val Leu Gly Ala Phe Glu Ser Ser Phe Trp His Met Gln Glu Lys Leu Gly Ile
            1225                1230                1235                1240
Val Gln Gly Ile Val Gly Ala Arg Asn Thr Ser Ala Ala Ser Thr Ala Gln Leu Val Glu
            1245                1250                1255                1260
Ala Thr Glu Glu Leu Arg Arg Glu Ile Gly Glu Ala Thr Glu His Leu Thr Gln Leu Glu
            1265                1270                1275                1280
Ala Asp Leu Thr Asp Val Gln Asp Glu Asn Phe Asn Ala Asn His Ala Leu Ser Gly Leu
            1285                1290                1295                1300
Glu Arg Asp Arg Leu Ala Leu Asn Leu Thr Leu Arg Gln Leu Asp Gln His Leu Asp Leu
            1305                1310                1315                1320
Leu Lys His Ser Asn Phe Leu Gly Ala Tyr Asp Ser Ile Arg His Ala His Ser Gln Ser
            1325                1330                1335                1340
Ala Glu Ala Glu Arg Arg Ala Asn Thr Ser Ala Leu Ala Val Pro Ser Pro Val Ser Asn
            1345                1350                1355                1360
Ser Ala Ser Ala Arg His Arg Thr Glu Ala Leu Met Asp Ala Gln Lys Glu Asp Phe Asn
            1365                1370                1375                1380
Ser Lys His Met Ala Asn Gln Arg Ala Leu Gly Lys Leu Ser Ala His Thr His Thr Leu
            1385                1390                1395                1400
Ser Leu Thr Asp Ile Asn Glu Leu Val Cys Gly Ala Pro Gly Asp Ala Pro Cys Ala Thr
            1405                1410                1415                1420
Ser Pro Cys Gly Gly Ala Gly Cys Arg Asp Glu Asp Gly Gln Pro Arg Cys Gly Gly Leu
            1425                1430                1435                1440
Ser Cys Asn Gly Ala Ala Ala Thr Ala Asp Leu Ala Leu Gly Arg Ala Arg His Thr Gln
            1445                1450                1455                1460
Ala Glu Leu Gln Arg Ala Leu Ala Glu Gly Gly Ser Ile Leu Ser Arg Val Ala Glu Thr
            1465                1470                1475                1480
Arg Arg Gln Ala Ser Glu Ala Gln Gln Arg Ala Gln Ala Ala Leu Asp Lys Ala Asn Ala
            1485                1490                1495                1500
Ser Arg Gly Gln Val Glu Gln Ala Asn Gln Glu Leu Gln Glu Leu Ile Gln Ser Val Lys
            1505                1510                1515                1520
Asp Phe Leu Asn Gln Glu Gly Ala Asp Pro Asp Ser Ile Glu Met Val Ala Thr Arg Val
            1525                1530                1535                1540
Leu Glu Leu Ser Ile Pro Ala Ser Ala Glu Gln Ile Gln His Leu Ala Gly Ala Ile Ala
            1545                1550                1555                1560
Glu Arg Val Arg Ser Leu Ala Asp Val Asp Ala Ile Leu Ala Arg Thr Val Gly Asp Val
            1565                1570                1575                1580
Arg Arg Ala Glu Gln Leu Leu Gln Asp Ala Arg Arg Ala Arg Ser Trp Ala Glu Asp Glu
            1585                1590                1595                1600
Lys Gln Lys Ala Glu Thr Val Gln Ala Ala Leu Glu Glu Ala Gln Arg Ala Gln Gly Ile
            1605                1610                1615                1620
Ala Gln Gly Ala Ile Arg Gly Ala Val Ala Asp Thr Arg Asp Thr Glu Gln Thr Leu Tyr
            1625                1630                1635                1640
Gln Val Gln Glu Arg Met Ala Gly Ala Glu Arg Ala Leu Ser Ser Ala Gly Glu Arg Ala
            1645                1650                1655                1660
Arg Gln Leu Asp Ala Leu Leu Glu Ala Leu Lys Leu Lys Arg Ala Gly Asn Ser Leu Ala
            1665                1670                1675                1680
Ala Ser Thr Ala Glu Glu Thr Ala Gly Ser Ala Gln Gly Arg Ala Gln Glu Ala Glu Gln
            1685                1690                1695                1700
Leu Leu Arg Gly Pro Leu Gly Asp Gln Tyr Gln Thr Val Lys Ala Leu Ala Glu Arg Lys
            1705                1710                1715                1720
Ala Gln Gly Val Leu Ala Ala Gln Ala Arg Ala Glu Gln Leu Arg Asp Glu Ala Arg Asp
            1725                1730                1735                1740
Leu Leu Gln Ala Ala Gln Asp Lys Leu Gln Arg Leu Gln Glu Leu Glu Gly Thr Tyr Glu
            1745                1750                1755                1760
```

Glu Asn Glu Arg Ala Leu Glu Ser Lys Ala Ala Gln Leu Asp Gly Leu Glu Ala Arg Met
1765 1770 1775 1780
Arg S r Val Leu Gln Ala Ile Asn Leu Gln Val Gln Ile Tyr Asn Thr Cys Gln
1785 1790 1795

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1607 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P02468

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Thr Gly Gly Gly Arg Ala Ala Leu Ala Leu Gln Pro Arg Gly Arg Leu Trp Pro Leu
1 5 10 15 20
Leu Ala Val Leu Ala Ala Val Ala Gly Cys Val Arg Ala Ala Met Asp Glu Cys Ala Asp
25 30 35 40
Glu Gly Gly Arg Pro Gln Arg Cys Met Pro Glu Phe Val Asn Ala Ala Phe Asn Val Thr
45 50 55 60
Val Val Ala Thr Asn Thr Cys Gly Thr Pro Pro Glu Glu Tyr Cys Val Gln Thr Gly Val
65 70 75 80
Thr Gly Val Thr Lys Ser Cys His Leu Cys Asp Ala Gly Gln Gln His Leu Gln His Gly
85 90 95 100
Ala Ala Phe Leu Thr Asp Tyr Asn Asn Gln Ala Asp Thr Thr Trp Trp Gln Ser Gln Thr
105 110 115 120
Met Leu Ala Gly Val Gln Tyr Pro Asn Ser Ile Asn Leu Thr Leu His Leu Gly Lys Ala
125 130 135 140
Phe Asp Ile Thr Tyr Val Arg Leu Lys Phe His Thr Ser Arg Pro Glu Ser Phe Ala Ile
145 150 155 160
Tyr Lys Arg Thr Arg Glu Asp Gly Pro Trp Ile Pro Tyr Gln Tyr Tyr Ser Gly Ser Cys
165 170 175 180
Glu Asn Thr Tyr Ser Lys Ala Asn Arg Gly Phe Ile Arg Thr Gly Gly Asp Glu Gln Gln
185 190 195 200
Ala Leu Cys Thr Asp Glu Phe Ser Asp Ile Ser Pro Leu Thr Gly Gly Asn Val Ala Phe
205 210 215 220
Ser Thr Leu Glu Gly Arg Pro Ser Ala Tyr Asn Phe Asp Asn Ser Pro Val Leu Gln Glu
225 230 235 240
Trp Val Thr Ala Thr Asp Ile Arg Val Thr Leu Asn Arg Leu Asn Thr Phe Gly Asp Glu
245 250 255 260
Val Phe Asn Glu Pro Lys Val Leu Lys Ser Tyr Tyr Tyr Ala Ile Ser Asp Phe Ala Val
265 270 275 280
Gly Gly Arg Cys Lys Cys Asn Gly His Ala Ser Glu Cys Val Lys Asn Glu Phe Asp Lys
285 290 295 300
Leu Met Cys Asn Cys Lys His Asn Thr Tyr Gly Val Asp Cys Glu Lys Cys Leu Pro Phe
305 310 315 320
Phe Asn Asp Arg Pro Trp Arg Arg Ala Thr Ala Glu Ser Ala Ser Glu Ser Leu Pro Cys
325 330 335 340
Asp Cys Asn Gly Arg Ser Gln Glu Cys Tyr Phe Asp Pro Glu Leu Tyr Arg Ser Thr Gly
345 350 355 360
His Gly Gly His Cys Thr Asn Cys Arg Asp Asn Thr Asp Gly Ala Lys Cys Glu Arg Cys
365 370 375 380
Arg Glu Asn Phe Phe Arg Leu Gly Asn Thr Glu Ala Cys Ser Pro Cys His Cys Ser Pro
385 390 395 400
Val Gly Ser Leu Ser Thr Gln Cys Asp Ser Tyr Gly Arg Cys Ser Cys Lys Pro Gly Val

405 410 415 420
Met Gly Asp Lys Cys Asp Arg Cys Gln Pro Gly Phe His Ser Leu Thr Glu Ala Gly Cys
425 430 435 440
Arg Pro Cys Ser Cys Asp Leu Arg Gly Ser Thr Asp Glu Cys Asn Val Glu Thr Gly Arg
445 450 455 460
Cys Val Cys Lys Asp Asn Val Glu Gly Phe Asn Cys Glu Arg Cys Lys Pro Gly Phe Phe
465 470 475 480
Asn Leu Glu Ser Ser Asn Pro Lys Gly Cys Thr Pro Cys Phe Cys Phe Gly His Ser Ser
485 490 495 500
Val Cys Thr Asn Ala Val Gly Tyr Ser Val Tyr Asp Ile Ser Ser Thr Phe Gln Ile Asp
505 510 515 520
Glu Asp Gly Trp Arg Val Glu Gln Arg Asp Gly Ser Glu Ala Ser Leu Glu Trp Ser Ser
525 530 535 540
Asp Arg Gln Asp Ile Ala Val Ile Ser Asp Ser Tyr Phe Pro Arg Tyr Phe Ile Ala Pro
545 550 555 560
Val Lys Phe Leu Gly Asn Gln Val Leu Ser Tyr Gly Gln Asn Leu Ser Phe Ser Phe Arg
565 570 575 580
Val Asp Arg Arg Asp Thr Arg Leu Ser Ala Glu Asp Leu Val Leu Glu Gly Ala Gly Leu
585 590 595 600
Arg Val Ser Val Pro Leu Ile Ala Gln Gly Asn Ser Tyr Pro Ser Glu Thr Thr Val Lys
605 610 615 620
Tyr Ile Phe Arg Leu His Glu Ala Thr Asp Tyr Pro Trp Arg Pro Ala Leu Ser Pro Phe
625 630 635 640
Glu Phe Gln Lys Leu Leu Asn Asn Leu Thr Ser Ile Lys Ile Arg Gly Thr Tyr Ser Glu
645 650 655 660
Arg Thr Ala Gly Tyr Leu Asp Asp Val Thr Leu Gln Ser Ala Arg Pro Gly Pro Gly Val
665 670 675 680
Pro Ala Thr Trp Val Glu Ser Cys Thr Cys Pro Val Gly Tyr Gly Gly Gln Phe Cys Glu
685 690 695 700
Thr Cys Leu Pro Gly Tyr Arg Arg Glu Thr Pro Ser Leu Gly Pro Tyr Ser Pro Cys Val
705 710 715 720
Leu Cys Thr Cys Asn Gly His Ser Glu Thr Cys Asp Pro Glu Thr Gly Val Cys Asp Cys
725 730 735 740
Arg Asp Asn Thr Ala Gly Pro His Cys Glu Lys Cys Ser Asp Gly Tyr Tyr Gly Asp Ser
745 750 755 760
Thr Leu Gly Thr Ser Ser Asp Cys Gln Pro Cys Pro Cys Pro Gly Gly Ser Ser Cys Ala
765 770 775 780
Ile Val Pro Lys Thr Lys Glu Val Val Cys Thr His Cys Pro Thr Gly Thr Ala Gly Lys
785 790 795 800
Arg Cys Glu Leu Cys Asp Asp Gly Tyr Phe Gly Asp Pro Leu Gly Ser Asn Gly Pro Val
805 810 815 820
Arg Leu Cys Arg Pro Cys Gln Cys Asn Asp Asn Ile Asp Pro Asn Ala Val Gly Asn Cys
825 830 835 840
Asn Arg Leu Thr Gly Glu Cys Leu Lys Cys Ile Tyr Asn Thr Ala Gly Phe Tyr Cys Asp
845 850 855 860
Arg Cys Lys Glu Gly Phe Phe Gly Asn Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Lys
865 870 875 880
Ala Cys Ala Cys Asn Pro Tyr Gly Thr Val Gln Gln Gln Ser Ser Cys Asn Pro Val Thr
885 890 895 900
Gly Gln Cys Gln Cys Leu Pro His Val Ser Gly Arg Asp Cys Gly Thr Cys Asp Pro Gly
905 910 915 920
Tyr Tyr Asn Leu Gln Ser Gly Gln Gly Cys Glu Arg Cys Asp Cys His Ala Leu Gly Ser
925 930 935 940
Thr Asn Gly Gln Cys Asp Ile Arg Thr Gly Gln Cys Glu Cys Gln Pro Gly Ile Thr Gly
945 950 955 960
Gln His Cys Glu Arg Cys Glu Thr Asn His Phe Gly Phe Gly Pro Glu Gly Cys Lys Pro
965 970 975 980
Cys Asp Cys His His Glu Gly Ser Leu Ser Leu Gln Cys Lys Asp Asp Gly Arg Cys Glu
985 990 995 1000
Cys Arg Glu Gly Phe Val Gly Asn Arg Cys Asp Gln Cys Glu Glu Asn Tyr Phe Tyr Asn
1005 1010 1015 1020
Arg Ser Trp Pro Gly Cys Gln Glu Cys Pro Ala Cys Tyr Arg Leu Val Lys Asp Lys Ala
1025 1030 1035 1040

Ala Glu His Arg Val Lys Leu Gln Glu Leu Glu Ser Leu Ile Ala Asn Leu Gly Thr Gly
1045 1050 1055 1060
Asp Asp Met Val Thr Asp Gln Ala Phe Glu Asp Arg Leu Lys Glu Ala Glu Arg Glu Val
1065 1070 1075 1080
Thr Asp Leu Leu Arg Glu Ala Gln Glu Val Lys Asp Val Asp Gln Asn Leu Met Asp Arg
1085 1090 1095 1100
Leu Gln Arg Val Asn Ser Ser Leu His Ser Gln Ile Ser Arg Leu Gln Asn Ile Arg Asn
1105 1110 1115 1120
Thr Ile Glu Glu Thr Gly Ile Leu Ala Glu Arg Ala Arg Ser Arg Val Glu Ser Thr Glu
1125 1130 1135 1140
Gln Leu Ile Glu Ile Ala Ser Arg Glu Leu Glu Lys Ala Lys Met Ala Ala Ala Asn Val
1145 1150 1155 1160
Ser Ile Thr Gln Pro Glu Ser Thr Gly Glu Pro Asn Asn Met Thr Leu Leu Ala Glu Glu
1165 1170 1175 1180
Ala Arg Arg Leu Ala Glu Arg His Lys Gln Glu Ala Asp Asp Ile Val Arg Val Ala Lys
1185 1190 1195 1200
Thr Ala Asn Glu Thr Ser Ala Glu Ala Tyr Asn Leu Leu Leu Arg Thr Leu Ala Gly Glu
1205 1210 1215 1220
Asn Gln Thr Ala Leu Glu Ile Glu Glu Leu Asn Arg Lys Tyr Glu Gln Ala Lys Asn Ile
1225 1230 1235 1240
Ser Gln Asp Leu Glu Lys Gln Ala Ala Arg Val His Glu Glu Ala Lys Arg Ala Gly Asp
1245 1250 1255 1260
Lys Ala Val Glu Ile Tyr Ala Ser Val Ala Gln Leu Thr Pro Val Asp Ser Glu Ala Leu
1265 1270 1275 1280
Glu Asn Glu Ala Asn Lys Ile Lys Lys Glu Ala Ala Asp Leu Asp Arg Leu Ile Asp Gln
1285 1290 1295 1300
Lys Leu Lys Asp Tyr Glu Asp Leu Arg Glu Asp Met Arg Gly Lys Glu His Glu Val Lys
1305 1310 1315 1320
Asn Leu Leu Glu Lys Gly Lys Ala Glu Gln Gln Thr Ala Asp Gln Leu Leu Ala Arg Ala
1325 1330 1335 1340
Asp Ala Ala Lys Ala Leu Ala Glu Glu Ala Ala Lys Lys Gly Arg Ser Thr Leu Gln Glu
1345 1350 1355 1360
Ala Asn Asp Ile Leu Asn Asn Leu Lys Asp Phe Asp Arg Arg Val Asn Asp Asn Lys Thr
1365 1370 1375 1380
Ala Ala Glu Glu Ala Leu Arg Arg Ile Pro Ala Ile Asn Arg Thr Ile Ala Glu Ala Asn
1385 1390 1395 1400
Glu Lys Thr Arg Glu Ala Gln Leu Ala Leu Gly Asn Ala Ala Ala Asp Ala Thr Glu Ala
1405 1410 1415 1420
Lys Asn Lys Ala His Glu Ala Glu Arg Ile Ala Ser Ala Val Gln Lys Asn Ala Thr Ser
1425 1430 1435 1440
Thr Lys Ala Asp Ala Glu Arg Thr Phe Gly Glu Val Thr Asp Leu Asp Asn Glu Val Asn
1445 1450 1455 1460
Gly Met Leu Arg Gln Leu Glu Glu Ala Glu Asn Glu Leu Lys Arg Lys Gln Asp Asp Ala
1465 1470 1475 1480
Asp Gln Asp Met Met Met Ala Gly Met Ala Ser Gln Ala Ala Gln Glu Ala Glu Leu Asn
1485 1490 1495 1500
Ala Arg Lys Ala Lys Asn Ser Val Ser Ser Leu Leu Ser Gln Leu Asn Asn Leu Leu Asp
1505 1510 1515 1520
Gln Leu Gly Gln Leu Asp Thr Val Asp Leu Asn Lys Leu Asn Glu Ile Glu Gly Ser Leu
1525 1530 1535 1540
Asn Lys Ala Lys Asp Glu Met Lys Ala Ser Asp Leu Asp Arg Lys Val Ser Asp Leu Glu
1545 1550 1555 1560
Ser Glu Ala Arg Lys Gln Glu Ala Ala Ile Met Asp Tyr Asn Arg Asp Ile Ala Glu Ile
1565 1570 1575 1580
Ile Lys Asp Ile His Asn Leu Glu Asp Ile Lys Lys Thr Leu Pro Thr Gly Cys Phe Asn
1585 1590 1595 1600
Thr Pro Ser Ile Glu Lys Pro
1605

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1609 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: PROTEIN

(ix) FEATURE:
(D) OTHER INFORMATION: AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF GENEBANK ACCESSION NUMBER P11047

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Met Arg Gly Ser His Arg Ala Ala Pro Ala Leu Arg Pro Arg Gly Arg Leu Trp Pro Val
1               5                  10                  15                  20
Leu Ala Val Leu Ala Ala Ala Ala Ala Ala Gly Cys Ala Gln Ala Ala Met Asp Glu Cys
            25                  30                  35                  40
Thr Asp Glu Gly Gly Arg Pro Gln Arg Cys Met Pro Glu Phe Val Asn Ala Ala Phe Asn
        45                  50                  55                  60
Val Thr Val Val Ala Thr Asn Thr Cys Gly Thr Pro Pro Glu Glu Tyr Cys Val Gln Thr
    65                  70                  75                  80
Gly Val Thr Gly Val Thr Lys Ser Cys His Leu Cys Asp Ala Gly Gln Pro His Leu Gln
                85                  90                  95                  100
His Gly Ala Ala Phe Leu Thr Asp Tyr Asn Asn Gln Ala Asp Thr Thr Trp Trp Gln Ser
            105                 110                 115                 120
Gln Thr Met Leu Ala Gly Val Gln Tyr Pro Ser Ser Ile Asn Leu Thr Leu His Leu Gly
        125                 130                 135                 140
Lys Ala Phe Asp Ile Thr Tyr Val Arg Leu Lys Phe His Thr Ser Arg Pro Glu Ser Phe
    145                 150                 155                 160
Ala Ile Tyr Lys Arg Thr Arg Glu Asp Gly Pro Trp Ile Pro Tyr Gln Tyr Tyr Ser Gly
                165                 170                 175                 180
Ser Cys Glu Asn Thr Tyr Ser Lys Ala Asn Arg Gly Phe Ile Arg Thr Gly Gly Asp Glu
            185                 190                 195                 200
Gln Gln Ala Leu Cys Thr Asp Glu Phe Ser Asp Phe Ser Pro Leu Thr Gly Gly Asn Val
        205                 210                 215                 220
Ala Phe Ser Thr Leu Glu Gly Arg Pro Ser Ala Tyr Asn Phe Asp Asn Ser Pro Val Leu
    225                 230                 235                 240
Gln Glu Trp Val Thr Ala Thr Asp Ile Arg Val Thr Leu Asn Arg Leu Asn Thr Phe Gly
                245                 250                 255                 260
Asp Glu Val Phe Asn Asp Pro Lys Val Leu Lys Ser Tyr Tyr Tyr Ala Ile Ser Asp Phe
            265                 270                 275                 280
Ala Val Gly Gly Arg Cys Lys Cys Asn Gly His Ala Ser Glu Cys Met Lys Asn Glu Phe
        285                 290                 295                 300
Asp Lys Leu Val Cys Asn Cys Lys His Asn Thr Tyr Gly Val Asp Cys Glu Lys Cys Leu
    305                 310                 315                 320
Pro Phe Phe Asn Asp Arg Pro Trp Arg Arg Ala Thr Ala Glu Ser Ala Ser Glu Cys Leu
                325                 330                 335                 340
Pro Cys Asp Cys Asn Gly Arg Ser Gln Glu Cys Tyr Phe Asp Pro Glu Leu Tyr Arg Ser
            345                 350                 355                 360
Thr Gly His Gly Gly His Cys Thr Asn Cys Gln Asp Asn Thr Asp Gly Ala His Cys Glu
        365                 370                 375                 380
Arg Cys Arg Glu Asn Phe Phe Arg Leu Gly Asn Asn Glu Ala Cys Ser Ser Cys His Cys
    385                 390                 395                 400
Ser Pro Val Gly Ser Leu Ser Thr Gln Cys Asp Ser Tyr Gly Arg Cys Ser Cys Lys Pro
                405                 410                 415                 420
Gly Val Met Gly Asp Lys Cys Asp Arg Cys Gln Pro Gly Phe His Ser Leu Thr Glu Ala
            425                 430                 435                 440
Gly Cys Arg Pro Cys Ser Cys Asp Pro Ser Gly Ser Ile Asp Glu Cys Asn Val Glu Thr
        445                 450                 455                 460
Gly Arg Cys Val Cys Lys Asp Asn Val Glu Gly Phe Asn Cys Glu Arg Cys Lys Pro Gly
    465                 470                 475                 480
Phe Phe Asn Leu Glu Ser Ser Asn Pro Arg Gly Cys Thr Pro Cys Phe Cys Phe Gly His
```

485 490 495 500
Ser Ser Val Cys Thr Asn Ala Val Gly Tyr Ser Val Tyr Ser Ile Ser Ser Thr Phe Gln
505 510 515 520
Ile Asp Glu Asp Gly Trp Arg Ala Glu Gln Arg Asp Gly Ser Glu Ala Ser Leu Glu Trp
525 530 535 540
Ser Ser Glu Arg Gln Asp Ile Ala Val Ile Ser Asp Ser Tyr Phe Pro Arg Tyr Phe Ile
545 550 555 560
Ala Pro Ala Lys Phe Leu Gly Lys Gln Val Leu Ser Tyr Gly Gln Asn Leu Ser Phe Ser
565 570 575 580
Phe Arg Val Asp Arg Arg Asp Thr Arg Leu Ser Ala Glu Asp Leu Val Leu Glu Gly Ala
585 590 595 600
Gly Leu Arg Val Ser Val Pro Leu Ile Ala Gln Gly Asn Ser Tyr Pro Ser Glu Thr Thr
605 610 615 620
Val Lys Tyr Val Phe Arg Leu His Glu Ala Thr Asp Tyr Pro Trp Arg Pro Ala Leu Thr
625 630 635 640
Pro Phe Glu Phe Gln Lys Leu Leu Asn Asn Leu Thr Ser Ile Lys Ile Arg Gly Thr Tyr
645 650 655 660
Ser Glu Arg Ser Ala Gly Tyr Leu Asp Asp Val Thr Leu Ala Ser Ala Arg Pro Gly Pro
665 670 675 680
Gly Val Pro Ala Thr Trp Val Glu Ser Cys Thr Cys Pro Val Gly Tyr Gly Gly Gln Phe
685 690 695 700
Cys Glu Met Cys Leu Ser Gly Tyr Arg Arg Glu Thr Pro Asn Leu Gly Pro Tyr Ser Pro
705 710 715 720
Cys Val Leu Cys Ala Cys Asn Gly His Ser Glu Thr Cys Asp Pro Glu Thr Gly Val Cys
725 730 735 740
Asn Cys Arg Asp Asn Thr Ala Gly Pro His Cys Glu Lys Cys Ser Asp Gly Tyr Tyr Gly
745 750 755 760
Asp Ser Thr Ala Gly Thr Ser Ser Asp Cys Gln Pro Cys Pro Cys Pro Gly Gly Ser Ser
765 770 775 780
Cys Ala Val Val Pro Lys Thr Lys Glu Val Val Cys Thr Asn Cys Pro Thr Gly Thr Thr
785 790 795 800
Gly Lys Arg Cys Glu Leu Cys Asp Asp Gly Tyr Phe Gly Asp Pro Leu Gly Arg Asn Gly
805 810 815 820
Pro Val Arg Leu Cys Arg Leu Cys Gln Cys Ser Asp Asn Ile Asp Pro Asn Ala Val Gly
825 830 835 840
Asn Cys Asn Arg Leu Thr Gly Glu Cys Leu Lys Cys Ile Tyr Asn Thr Ala Gly Phe Tyr
845 850 855 860
Cys Asp Arg Cys Lys Asp Gly Phe Phe Gly Asn Pro Leu Ala Pro Asn Pro Ala Asp Lys
865 870 875 880
Cys Lys Ala Cys Asn Cys Asn Pro Tyr Gly Thr Met Lys Gln Gln Ser Ser Cys Asn Pro
885 890 895 900
Val Thr Gly Gln Cys Glu Cys Leu Pro His Val Thr Gly Gln Asp Cys Gly Ala Cys Asp
905 910 915 920
Pro Gly Phe Tyr Asn Leu Gln Ser Gly Gln Gly Cys Glu Arg Cys Asp Cys His Ala Leu
925 930 935 940
Gly Ser Thr Asn Gly Gln Cys Asp Ile Arg Thr Gly Gln Cys Glu Cys Gln Pro Gly Ile
945 950 955 960
Thr Gly Gln His Cys Glu Arg Cys Glu Val Asn His Phe Gly Phe Gly Pro Glu Gly Cys
965 970 975 980
Lys Pro Cys Asp Cys His Pro Glu Gly Ser Leu Ser Leu Gln Cys Lys Asp Asp Gly Arg
985 990 995 1000
Cys Glu Cys Arg Glu Gly Phe Val Gly Asn Arg Cys Asp Gln Cys Glu Glu Asn Tyr Phe
1005 1010 1015 1020
Tyr Asn Arg Ser Trp Pro Gly Cys Gln Glu Cys Pro Ala Cys Tyr Arg Leu Val Lys Asp
1025 1030 1035 1040
Lys Val Ala Asp His Arg Val Lys Leu Gln Glu Leu Glu Ser Leu Ile Ala Asn Leu Gly
1045 1050 1055 1060
Thr Gly Asp Glu Met Val Thr Asp Gln Ala Phe Glu Asp Arg Leu Lys Glu Ala Glu Arg
1065 1070 1075 1080
Glu Val Met Asp Leu Leu Arg Glu Ala Gln Asp Val Lys Asp Val Asp Gln Asn Leu Met
1085 1090 1095 1100
Asp Arg Leu Gln Arg Val Asn Asn Thr Leu Ser Ser Gln Ile Ser Arg Leu Gln Asn Ile
1105 1110 1115 1120

```
Arg Asn Thr Ile Glu Glu Thr Gly Asn Leu Ala Glu Gln Ala Arg Ala His Val Glu Asn
                1125                1130                1135                1140
Thr Glu Arg Leu Ile Glu Ile Ala Ser Arg Glu Leu Glu Lys Ala Lys Val Ala Ala Ala
                1145                1150                1155                1160
Asn Val Ser Val Thr Gln Pro Glu Ser Thr Gly Asp Pro Asn Asn Met Thr Leu Leu Ala
                1165                1170                1175                1180
Glu Glu Ala Arg Lys Leu Ala Glu Arg His Lys Gln Glu Ala Asp Asp Ile Val Arg Val
                1185                1190                1195                1200
Ala Lys Thr Ala Asn Asp Thr Ser Thr Glu Ala Tyr Asn Leu Leu Leu Arg Thr Leu Ala
                1205                1210                1215                1220
Gly Glu Asn Gln Thr Ala Phe Glu Ile Glu Glu Leu Asn Arg Lys Tyr Glu Gln Ala Lys
                1225                1230                1235                1240
Asn Ile Ser Gln Asp Leu Glu Lys Gln Ala Ala Arg Val His Glu Glu Ala Lys Arg Ala
                1245                1250                1255                1260
Gly Asp Lys Ala Val Glu Ile Tyr Ala Ser Val Ala Gln Leu Ser Pro Leu Asp Ser Glu
                1265                1270                1275                1280
Thr Leu Glu Asn Glu Ala Asn Asn Ile Lys Met Glu Ala Glu Asn Leu Glu Gln Leu Ile
                1285                1290                1295                1300
Asp Gln Lys Leu Lys Asp Tyr Glu Asp Leu Arg Glu Asp Met Arg Gly Lys Glu Leu Glu
                1305                1310                1315                1320
Val Lys Asn Leu Leu Glu Lys Gly Lys Thr Glu Gln Gln Thr Ala Asp Gln Leu Leu Ala
                1325                1330                1335                1340
Arg Ala Asp Ala Ala Lys Ala Leu Ala Glu Glu Ala Ala Lys Lys Gly Arg Asp Thr Leu
                1345                1350                1355                1360
Gln Glu Ala Asn Asp Ile Leu Asn Asn Leu Lys Asp Phe Asp Arg Arg Val Asn Asp Asn
                1365                1370                1375                1380
Lys Thr Ala Ala Glu Glu Ala Leu Arg Lys Ile Pro Ala Ile Asn Gln Thr Ile Thr Glu
                1385                1390                1395                1400
Ala Asn Glu Lys Thr Arg Glu Ala Gln Gln Ala Leu Gly Ser Ala Ala Ala Asp Ala Thr
                1405                1410                1415                1420
Glu Ala Lys Asn Lys Ala His Glu Ala Glu Arg Ile Ala Ser Ala Val Gln Lys Asn Ala
                1425                1430                1435                1440
Thr Ser Thr Lys Ala Glu Ala Glu Arg Thr Phe Ala Glu Val Thr Asp Leu Asp Asn Glu
                1445                1450                1455                1460
Val Asn Asn Met Leu Lys Gln Leu Gln Glu Ala Glu Lys Glu Leu Lys Arg Lys Gln Asp
                1465                1470                1475                1480
Asp Ala Asp Gln Asp Met Met Met Ala Gly Met Ala Ser Gln Ala Ala Gln Glu Ala Glu
                1485                1490                1495                1500
Ile Asn Ala Arg Lys Ala Lys Asn Ser Val Thr Ser Leu Leu Ser Ile Ile Asn Asp Leu
                1505                1510                1515                1520
Leu Glu Gln Leu Gly Gln Leu Asp Thr Val Asp Leu Asn Lys Leu Asn Glu Ile Glu Gly
                1525                1530                1535                1540
Thr Leu Asn Lys Ala Lys Asp Glu Met Lys Val Ser Asp Leu Asp Arg Lys Val Ser Asp
                1545                1550                1555                1560
Leu Glu Asn Glu Ala Lys Lys Gln Glu Ala Ala Ile Met Asp Tyr Asn Arg Asp Ile Glu
                1565                1570                1575                1580
Glu Ile Met Lys Asp Ile Arg Asn Leu Glu Asp Ile Arg Lys Thr Leu Pro Ser Gly Cys
                1585                1590                1595                1600
Phe Asn Thr Pro Ser Ile Glu Lys Pro
                1605
```